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Applications of Lipase as a Catalyst for Stereoselective Reactions in Organic Solvent

MINORU INAGAKI

1991

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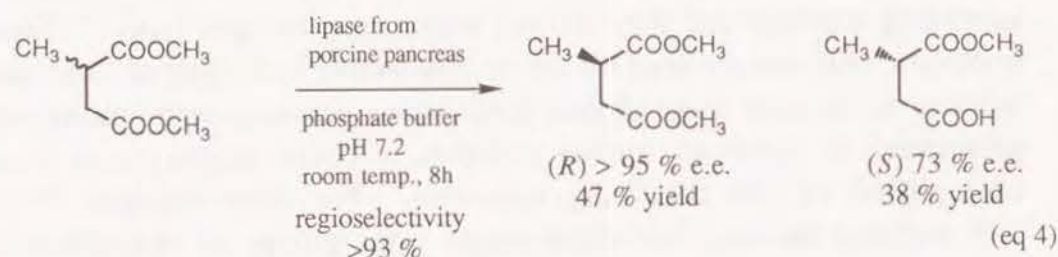
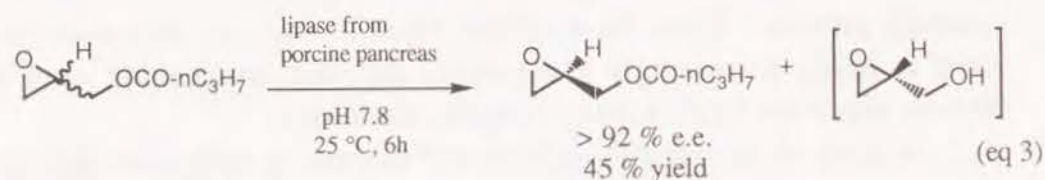
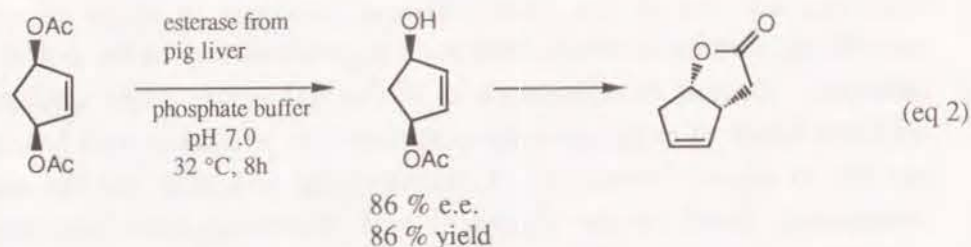
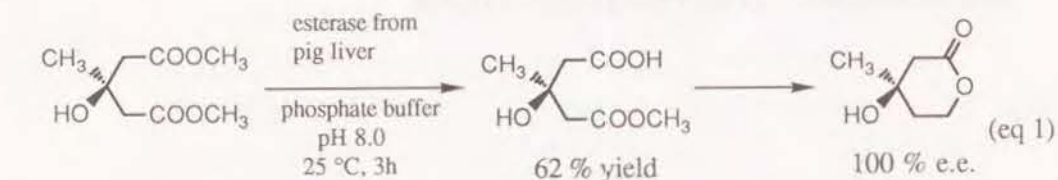
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GENERAL INTRODUCTION

Enzymes are one of the most efficient catalysts in terms of substrate-specificity, stereoselectivity, and even regioselectivity in the reactions they catalyze. Recent development in biological science has enabled many different kinds of enzymes to be commercially available with low price for the use in organic synthesis. Environmental concerns and the regulatory constraints faced in the chemical and pharmaceutical industries have spurred the hope that biological methods may offer a clean and mild synthetic process. Thus, the synthetic value of enzymes as a catalyst *in vitro* is being increasingly recognized, and this challenging area has become important field of today's organic chemistry.

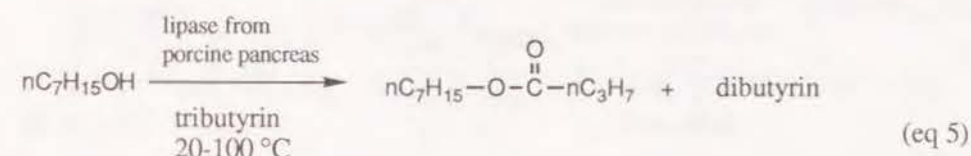
A kind of the synthetically useful enzyme is hydrolase including esterases, proteases, and lipases, because they require no cofactor for their catalyzing reactions and they showed broad substrate specificity.¹⁾ These hydrolytic enzymes are used for the stereoselective hydrolysis of esters and amides. In the early stage of their applications, reactions were exclusively performed in aqueous buffer solution. These applications were exemplified by the following reactions. Pig liver esterase (PLE) differentiated between the enantiotopic ester groups of σ -symmetric dicarboxylate to give the half-ester that was converted into optically pure mevalonolactone (eq 1).²⁾ PLE also hydrolyzed one of the two acetate groups of 1,4-*cis*-diacetoxycyclopentene stereoselectively, and the half-ester thus obtained was converted into a chiral synthon for the synthesis of prostaglandins (eq 2).³⁾ Porcine pancreatic lipase (PPL) was used for the kinetic resolution of racemic glycidol esters (eq 3).⁴⁾

The regioselectivity as well as the stereoselectivity of PPL-catalyzed hydrolysis was exploited by Guibé-Jampel et al.⁵⁾ The dimethyl ester of racemic 2-methylsuccinic acid was hydrolyzed by PPL. After the reaction, optical purity of the (*R*)-diester recovered in 47 % chemical yield was 95 % e.e. (eq 4). This result showed that PPL exclusively hydrolyzed the less hindered ester group of (*S*)-enantiomer.

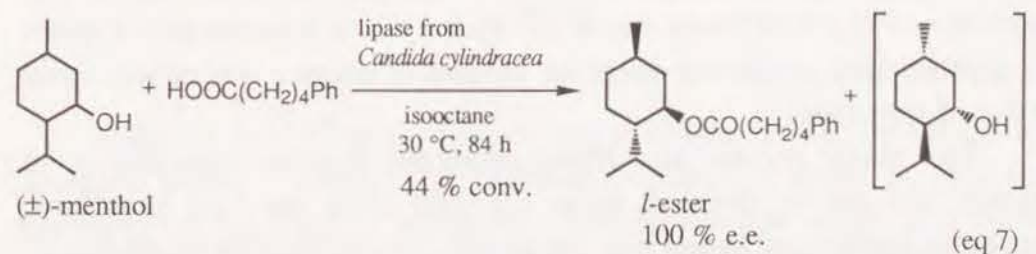
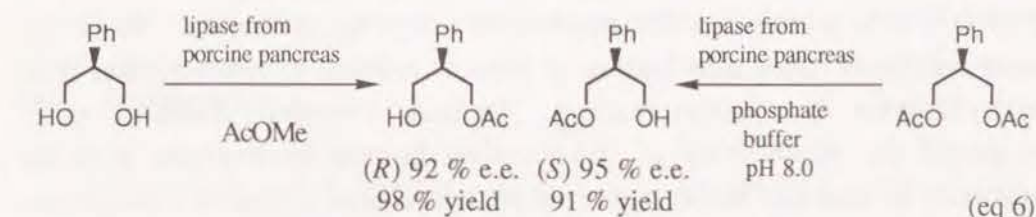


So far, the enzymatic process has been conducted mostly in aqueous solution, because it was believed that enzymes functioned only in an aqueous environment. One of the disadvantages of the use of lipase in aqueous buffer is that most starting materials for organic synthesis are non polar and usually insoluble in aqueous buffer. Actually, the applications in aqueous buffer are limited by the poor solubility of the non-polar substrates in water. Then, the water-miscible solvents such as methanol, ethanol, acetone, DMF, DMSO, and THF were added in order to dissolve the substrates, but these solvents were harmful to enzymatic activities under some conditions.^{6, 7, 8)} On the other hand, it was found that lipases suspended in pure and water-immiscible organic solvents were more stable. In 1984, Klibanov et al.⁹⁾ reported that lipases suspended in water-immiscible solvents such as hexane and isooctane remained a catalytic

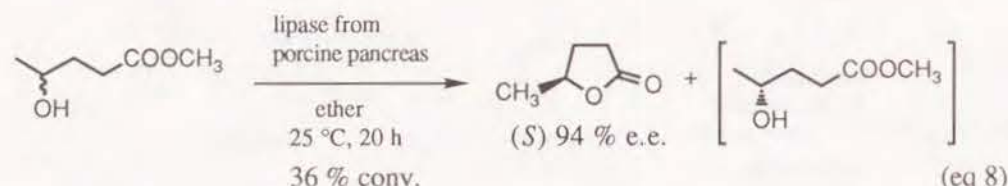
activity and catalyzed the transesterification reaction between 1-heptanol and tributyrin (eq 5). This observation opened up a new area of applications of lipase as a catalyst to organic synthesis. Other advantage of the use of lipase in organic solvent is that PPL is thermally stable in an organic solvent even at 100 °C, whereas the lipase loses its activity instantaneously in aqueous medium.⁹⁾



Lipases in organic solvent have realized new reactions such as ester-form and transesterification which were impossible in aqueous system. Ghisalba et al.¹⁰⁾ succeeded in the asymmetric induction by PPL-catalyzed acylation of the diols with σ -symmetry in organic solvents as well as PPL-catalyzed hydrolysis of their diacetates in aqueous solution (eq 6). In these two reactions, PPL showed the preference to the *pro-R* group of the enantiotopic hydroxy- and ester-groups. Both of the enantiomers of the monoacetate were obtained with high optical yield in combination of the two PPL-catalyzed reactions. Fukui et al.⁷⁾ resolved (\pm)-menthol by the ester formation with 5-phenylvaleric acid in isooctane using a lipase from *Candida cylindracea* (eq 7).



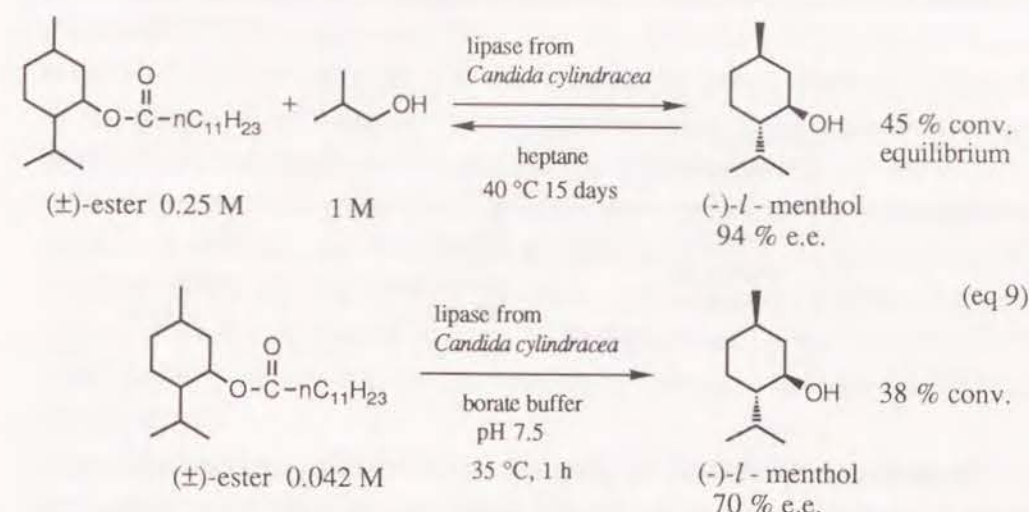
The lipase selectively acylated *l*-menthol to give optically pure *l*-menthyl ester. Gutman et al.¹¹⁾ obtained a chiral five-membered lactone with 94 % e.e. from racemic methyl 4-hydroxypentanoate by means of lipase-catalyzed lactonization (eq 8).



In the above examples, lipase was immobilized on macromolecular support and suspended in organic solvent. Immobilization of lipase has been devised to obtain a lipase-preparation having high catalytic activity even in a heterogeneous system.^{12, 13, 14, 15)} Wisdom et al. reported that a diatomaceous earth, Hyflo Super-Cel, was a superior support for the immobilization of lipase in order to exhibit the activity for the transesterification between fatty acid and triglyceride.¹⁵⁾ Insolubility of lipase in organic solvent permits easy separation and recovery from the reaction mixture after the reaction. Fukui et al. recovered the immobilized lipase from the reaction mixture (eq 7) and reused it 10 times without any loss of the activity.⁷⁾

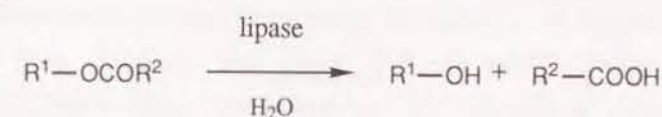
In this way, reactions catalyzed by lipase in organic solvent have become one of the most attractive areas for organic chemists who try to develop a new stereoselective reaction for organic synthesis. However, lipase-catalyzed transesterification in organic solvent is much slower than the hydrolysis in aqueous buffers. In fact, Triantaphylides et al.¹⁶⁾ compared the alcoholysis of (\pm)-menthyl laurate in heptane with its hydrolysis in aqueous buffer catalyzed by lipase from *Candida cylindracea* (eq 9). They observed that the initial rate of the alcoholysis in heptane was much slower (1 / 9700) than that of the hydrolysis in aqueous buffer under the experimental conditions where the amount of enzyme was almost equal to that of substrate.

The major reason why lipase-catalyzed reaction was slower in organic solvent is derived from the fact that the two reactions, transesterification and hydrolysis, are an equilibrium reaction in nature.

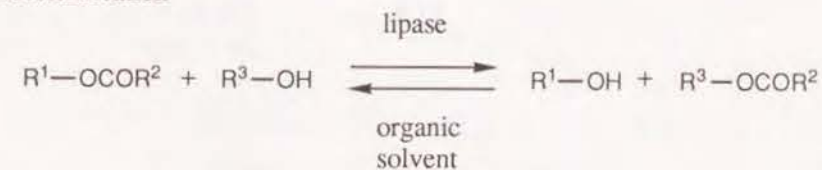


In hydrolytic reaction, solvent water is one of the substrates and its concentration is as high as 55.5 M. Such high concentration of water not only promotes the reaction toward its equilibrium state but also shifts the equilibrium far to the hydrolysis side. Hence the reaction is practically irreversible. In transesterification reaction, on the other, the concentration of the reactants are not so high, and the reaction is affected by mass action; the reverse reaction becomes significant compared to the forward reaction.

Hydrolysis

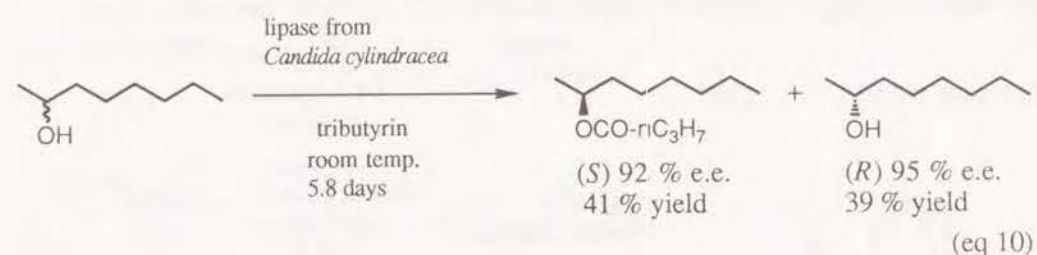


Transesterification

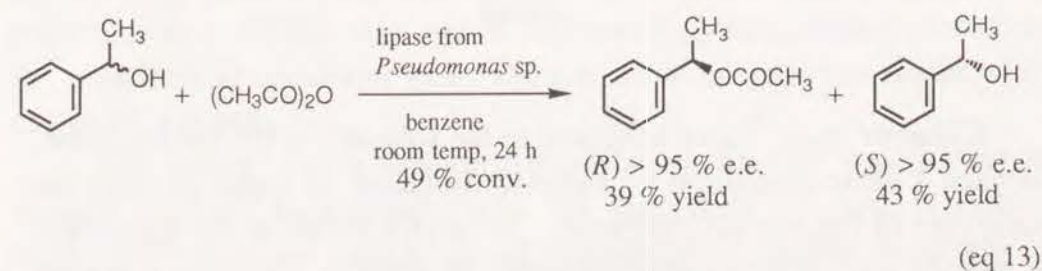
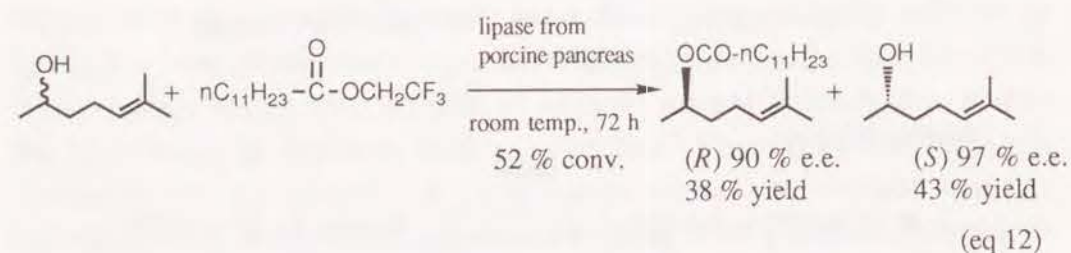
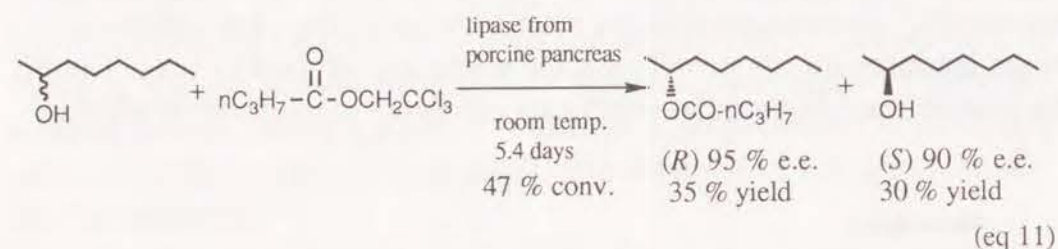


Klibanov et al.¹⁷⁾ used tributyrin as the solvent (eq 10) for the lipase-catalyzed kinetic resolution of racemic 2-octanol in order to shift the equilibrium to the product formation. Tributyrin works as the acyl-donor and attained high substrate concentration. In addition, tributyrin is a good

acyl-donor for lipase because it is one of triglycerides, natural substrate of lipase.



In ordinary synthesis, to prepare an ester from an alcohol, active acylating reagents, such as acid anhydrides and acyl halides are usually employed. The same is true in lipase-catalyzed reactions. Activated esters such as 2,2,2-trichloroethyl butyrate (eq 11),¹⁸⁾ 2,2,2-trifluoroethyl laurate (eq 12),¹⁹⁾ and acetic anhydride (eq 13)²⁰⁾ were tried for the lipase-catalyzed acylation of alcohols in organic solvent.



These attempts did not always give satisfactory results: the reactions of trihaloethyl esters were still slow and the acid formed inevitably from the anhydride was reported to inactivate lipase in other report.²¹⁾ Therefore, development of new acylating reagents have been required for the lipase-catalyzed transesterification reactions in organic solvent.

In chapter I, the lipase-catalyzed acylation of 1-hexanol was conducted with various enol esters to find an effective acylating reagent for transesterification. By using enol esters, the acylation became irreversible and quantitative in organic solvent. Then the reaction conditions optimized were applied to the kinetic resolution of racemic 2-halo-1-arylethanol (halohydrins).

In chapter II, phenol was successfully acylated by using enol esters. This reaction system was extended to the kinetic resolution of racemic mixture of axially dissymmetrical [1,1'-binaphthyl]-2,2'-diol (binaphthol).

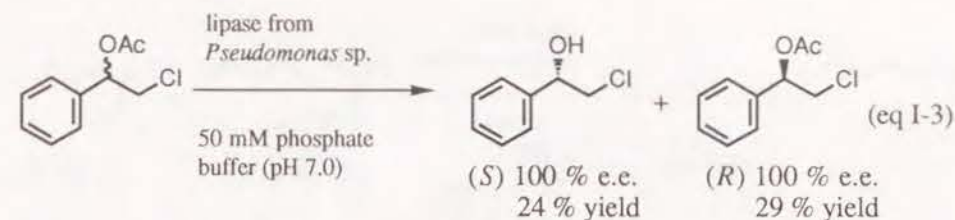
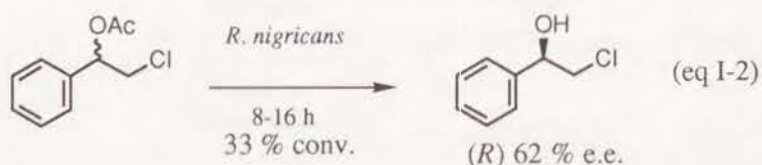
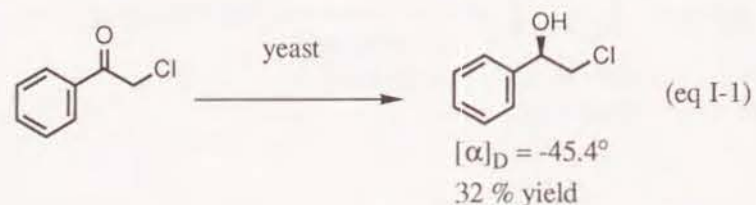
One of the advantages of conducting enzymatic reaction in organic solvent is that the compounds unstable in aqueous buffer can be used as a substrate. In chapter III, racemic mixture of 1-cyano-1-phenylmethanol (mandelonitrile) which are susceptible to decomposition in aqueous medium, was successfully resolved by lipase-catalyzed acetylation using isopropenyl acetate as an acylating reagent.

Extension of this reaction was accomplished in chapter IV: cyanohydrins were prepared by base-catalysts from aldehydes and 2-cyano-2-propanol, followed by the lipase-catalyzed stereoselective acetylation to yield the optically active cyanohydrin acetates. Due to the reversible nature of the base-catalyzed cyanohydrin formation, *in situ* racemization of cyanohydrins was attained and gave the optically active cyanohydrin acetates much more than the theoretical chemical yield of kinetic resolution, 50 %.

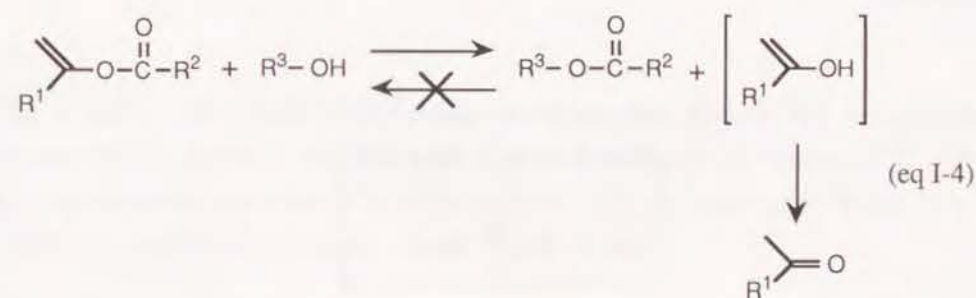
Irreversible and Highly Enantioselective Acylation of Racemic 2-Halo-1-arylethanols with Enol Esters Catalyzed by Lipase in Organic Solvent

I-1 INTRODUCTION

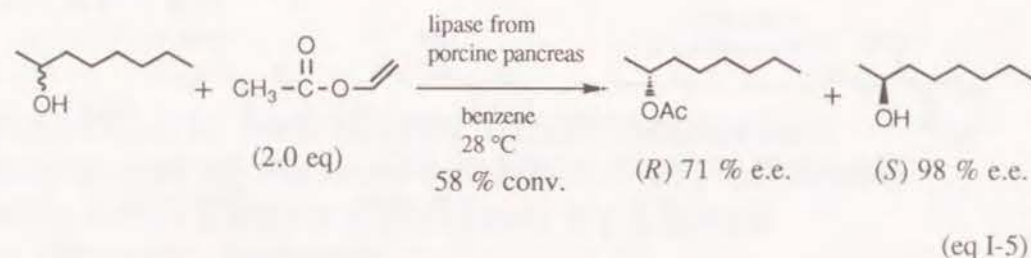
The author focused his attention on the kinetic resolution of racemic 2-halo-1-arylethanols (halohydrins) on the basis of the lipase-catalyzed transesterification in organic solvents. Optically active halohydrins are versatile intermediates for the synthesis of the compounds of pharmaceutical interest, such as an adrenergic agent epinephrine.^{22, 23, 24} Several biochemical approaches have been reported for the preparation of optically active halohydrins involving the asymmetric reduction of α -halogenated acetophenones (eq I-1)²⁵ and kinetic resolution by stereoselective hydrolysis of the corresponding racemic esters using microorganisms (eq I-2)²⁶ or lipases (eq I-3).²⁷ However, lipase-catalyzed stereoselective acylation of racemic halohydrins in organic solvent has not been reported yet.



Lipase-catalyzed transesterification reaction is reversible and often requires long reaction time and a large excess of esters as the acyl donor in order to achieve a reasonable degree of conversion. To find practical solutions to this problem, acylating agents were searched for the lipase-catalyzed transesterification. Enol esters are an active ester due to its characteristic structure of alcohol moiety. Once enol ester reacts with an alcohol, the enol liberated spontaneously isomerizes to ketone or aldehyde which no longer participates in the reaction; the reaction is irreversible (eq I-4).

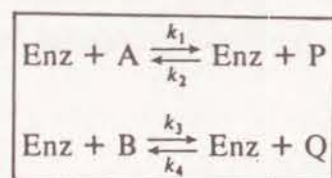


Enol esters have been used to the non-enzymatic acylation of sterically hindered alcohols including *tert*-butanol and *tert*-amyl alcohol in the presence of acid catalyst to give their acetate in high chemical yields.²⁸ Recently, Wong et al.²⁹ applied enol esters to the enzymatic acylation of σ -symmetric diols and kinetic resolution of racemic alcohols. They compared vinyl acetate with ethyl acetate for the PPL-catalyzed acylation of racemic 2-octanol (eq I-5). The reaction of vinyl acetate was 55 times faster than that of ethyl acetate.



Although enol esters were then used to the kinetic resolution of racemic alcohols,^{30, 31)} irreversibility of the reaction has not been clearly demonstrated yet.

Introducing an irreversible process is expected to improve not only the chemical yield, but also the optical yield in the kinetic resolution of racemic alcohols. Sih et al.³²⁾ demonstrated that kinetic resolution become most efficient when the process is irreversible. They theoretically analyzed an enzyme-catalyzed kinetic resolution in which an enantiomeric pair of the substrates, A and B, is converted into the product, P and Q, in a reversible process with an equilibrium constant K , and presented the following equation.



$$\frac{\ln [1 - (1 + K)(c + ees[1 - c])]}{\ln [1 - (1 + K)(c - ees[1 - c])]} = E \left(\begin{array}{c} \text{SUBSTRATE} \\ \text{FRACTION} \end{array} \right)$$

$$\left(K = \frac{k_2}{k_1} = \frac{k_4}{k_3} = \frac{A}{P} = \frac{B}{Q} \quad c = 1 - \frac{A+B}{A_0+B_0}; \quad c_{es} = \frac{B-A}{A+B} \right)$$

This equation shows that the enantiomeric excess of the product depends not only on the conversion c , but also on the equilibrium constant K .

Fig. I-1 is a graphic illustration of such situation when the stereoselection of the enzyme is high enough ($E = 100$).

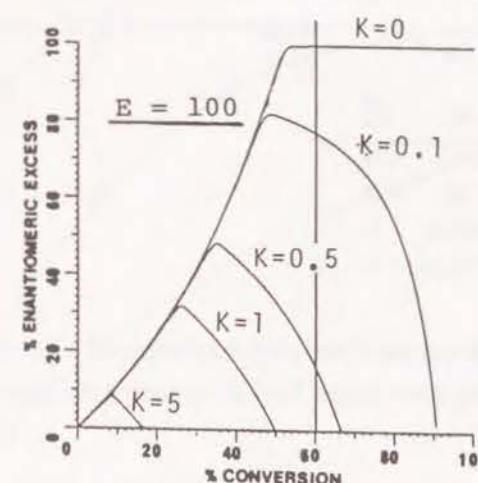


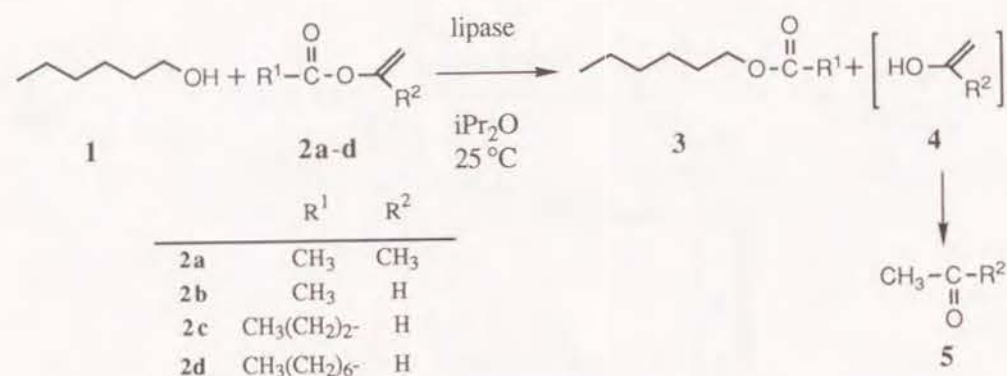
Fig. 1-1 Expression of the percentage enantiomeric excess (e.e.) of substrate fraction as a function of the percentage conversion at values of E is 100. These curves were computer generated from the above equation when the values of K were 0, 0.1, 0.5, 1, and 5. [Lit.³²⁾ C. J. Sih et al. *J. Am. Chem. Soc.*, **1987**, *109*, 2812].

The smaller the equilibrium constant K is, the higher the enantiomeric excess of the product becomes at a specific degree of conversion. When the equilibrium constant K is zero (irreversible process), the most efficient resolution can be expected.

I-2 RESULTS AND DISCUSSION

Lipase-Catalyzed Irreversible Acylation of 1-Hexanol Using Enol Esters as Acylating Reagent

In order to examine the potential of enol esters as an acylating reagent, the acylation of 1-hexanol (**1**) was conducted with an equimolar amount of enol esters **2a-d** in the presence of lipase in dry diisopropyl ether (Scheme I-1).



Scheme I-1 Lipase-Catalyzed Acylation of 1-Hexanol (1) Using Enol Esters 2a-d as Acylating Reagent

Non-polar and aprotic solvents such as hexane, cyclohexane, and ether were found suitable for the present reaction as indicated previously by Klivanov⁸⁾ and Fukui.⁷⁾ Diisopropyl ether was selected as the reaction medium throughout the present study because in this solvent lipase showed high catalytic activity and most of synthetic substrates were soluble. Among seven of the commercially available lipase preparations tested, lipase from *Pseudomonas* sp. M-12-33 (Amano Pharm. Co., Ltd.) was found to catalyze this reaction most efficiently and used in the following experiment.

The time course of the reactions is shown in Fig. I-2. 1-Hexanol (1) was effectively converted into the corresponding 1-hexyl esters 3 by lipase catalysis using enol esters 2a-d. The reaction using vinyl acetate (2b) reached 100 % conversion in 5 h. The other reactions using 2a, 2c-d also reached 100 % conversion within 22 h. No other product and no starting material was appeared during a prolonged incubation of the reaction mixture after the reaction completed. This indicates that the reaction of enol esters 2 and 1-hexanol (1) was quantitative and irreversible. Since acetaldehyde and acetone are volatile, hexyl esters 3 are easily isolated by evaporation. On the other hand, the reaction using 2,2,2-trichloroethyl acetate, one of the active esters so far used for the lipase-catalyzed acylation of alcohols,^{18, 19, 33, 34, 35)} proceeded slowly and reached 97 % conversion at last after 93 h.

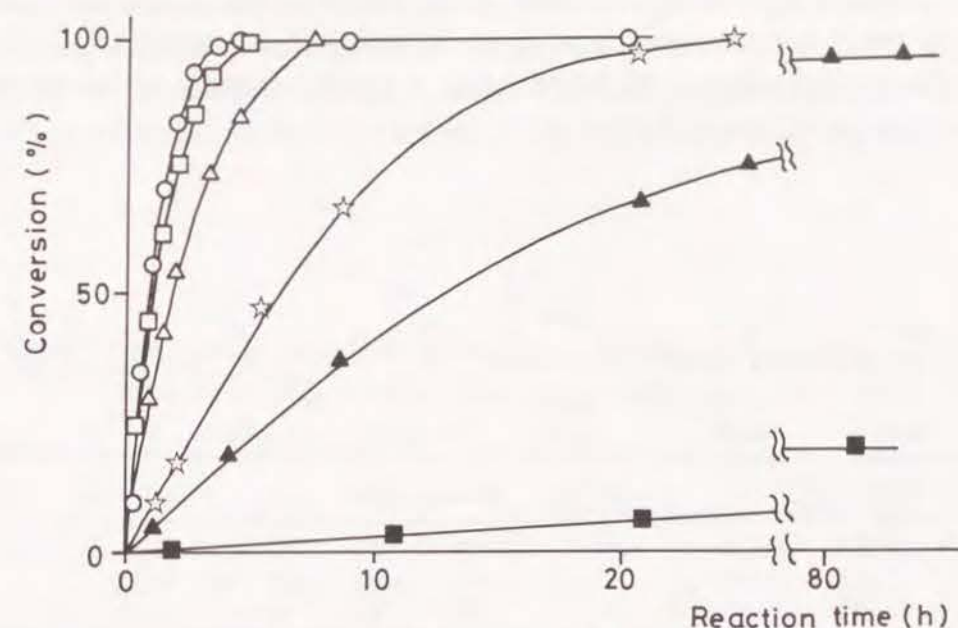
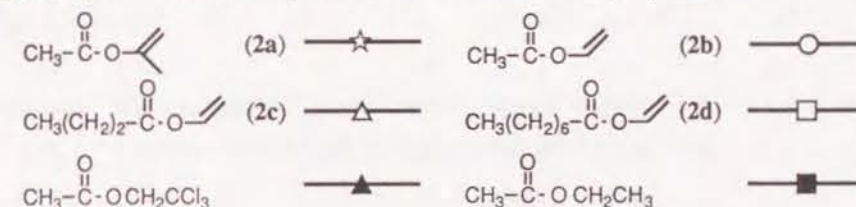


Fig. I-2 Time course of the lipase-catalyzed acylation of 1-hexanol (1) using enol esters 2a-d, trichloroethyl acetate, and ethyl acetate. 1-Hexanol (1) (1.02 g, 10.0 mmol) was allowed to react with acylating reagent (10.5 mmol) in the presence of a lipase (500 mg) from *Pseudomonas* sp. M-12-33 (Amano) in diisopropyl ether (20 mL) at 25 ± 1 °C. The conversion was determined by GLC from the decrease of 1-hexanol compared with the internal standard, ethylbenzene.

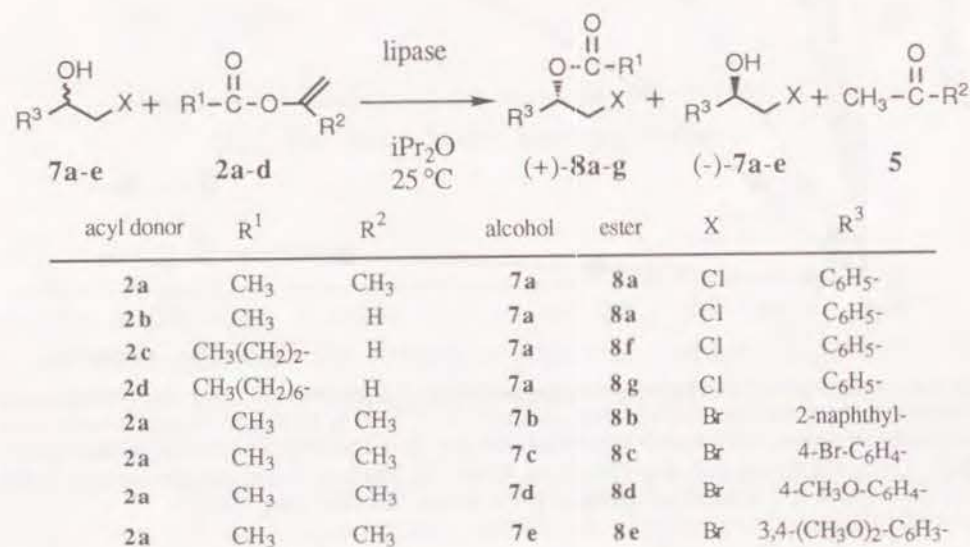


The reaction using ethyl acetate reached only 26 % conversion in the same reaction period. These results confirmed the potential of enol esters as mild, efficient, and irreversible acylating reagent for lipase-catalyzed reactions.

Lipase-Catalyzed Kinetic Resolution of Racemic 2-Halo-1-arylethanol Catalyzed by Lipase in Organic Solvents

Based on the above observations, enol esters were applied to the kinetic resolution of racemic 2-halo-1-arylethanol (halohydrins). Halohydrins

7a-e were prepared by NaBH₄-reduction of the corresponding haloketones **6a-e**. Racemic halohydrins was allowed to react with two equivalent moles of enol esters **2** in the presence of lipase from *Pseudomonas* sp. M-12-33 in dry diisopropyl ether at 25 °C (Scheme I-2). The results of the kinetic resolution are shown in Table I-1.



Scheme I-2 Lipase-Catalyzed Kinetic Resolution of Racemic 2-Halo-1-arylethanol **7a-e** Using Enol Esters **2a-d** as Acylating Reagent

The reactions virtually ceased when a half mole of racemic halohydrins **7** was consumed and an excess amount of acylating reagents **2** was still remained. This indicated that the stereoselectivity of the lipase was very strict. After the lipase powder was removed by filtration, evaporation of solvent and chromatography on silica gel gave the product esters **8** and the unreacted alcohols **7** in high chemical yields.

The reaction of **7a** with vinyl acetate (**2b**) terminated after 5 h; the reaction with **2b** was much faster than those with isopropenyl acetate (**2a**), vinyl butyrate (**2c**), and vinyl octanoate (**2d**). The absolute configurations and enantiomeric excesses of **7a**, **8a**, **8f**, and **8g** were determined by comparing their optical rotation with the reported values.²⁷⁾ Kinetic resolution of racemic **7a** with vinyl acetate (**2b**) gave (*S*)-(+)-**7a** at 94 %

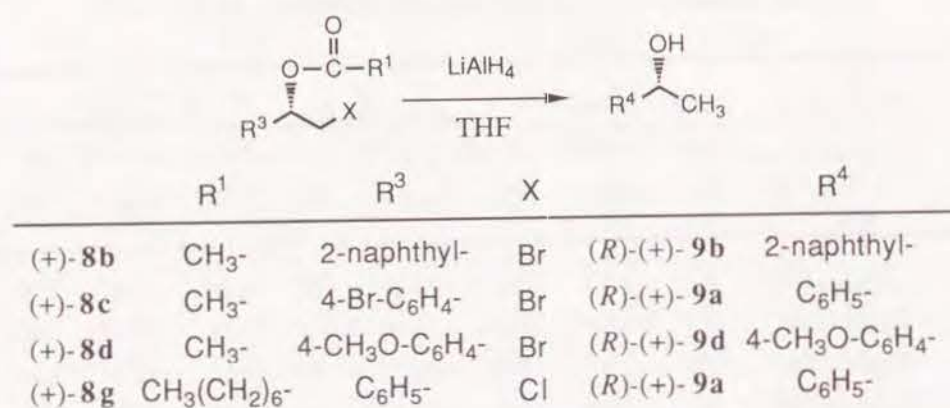
e.e. and (*R*)-(-)-**8a** at 93 % e.e. Similarly, kinetic resolution of **7a** with vinyl butyrate (**2c**) gave (*R*)-(-)-**7a** at 96 % e.e. and (*S*)-(+)-**8f** at 97 % e.e. and with vinyl octanoate (**2d**) (*R*)-(-)-**7a** at 92 % e.e. and (*S*)-(+)-**8g** at 96 % e.e. in excellent chemical yields. The stereoselectivity of the reaction was independent to the chain length of the acyl groups of enol esters.

Table I-1 Stereoselective Acylation of Racemic 2-Halo-1-arylethanol **7a-e** with Enol Esters **2a-d** Catalyzed by a Lipase from *Pseudomonas* sp. M-12-33^a

sub- strate	enol ester 2		react. time (h)	conver- sion ^b (%)	(S)-ester 8		(R)-alcohol 7	
	R ²	R ³			yield ^c (%)	[α] _D ²⁵ (deg.)	e.e. (%)	yield ^c (%)
								[α] _D ²⁵ (deg.)
								e.e. (%)
7a	CH ₃	CH ₃	17	52	52	+73.2 ^d	92 ^e	44
7a	CH ₃	H	5	51	52	+74.0 ^d	93 ^e	51
7a	nC ₃ H ₇	H	24	52	49	+66.2 ^g	97 ^e	46
7a	nC ₇ H ₁₅	H	24	50	49	+47.9 ^g	96 ^h	47
7b	CH ₃	CH ₃	38	50	48	+70.0 ⁱ	95 ^h	50
7c	CH ₃	CH ₃	26	50	48	+56.6 ^j	95 ^m	51
7d	CH ₃	CH ₃	30	49	48	+73.4 ^o	93 ^m	50
7e^p	CH ₃	CH ₃	42	50	47	+83.2 ^o	97 ^m	46

^a Conditions: substrate **7** (4.0-13 mmol), enol ester **2** (8.0-26 mmol), dry lipase from *Pseudomonas* sp. M-12-33 (2.0-6.5 g), dry diisopropyl ether (20 - 65 mL), 25 °C. ^b Determined by HPLC (hexane/AcOEt). ^c Isolated yield based on racemic **7**. ^d c 2.0, acetone. ^e Determined by comparison of the observed specific rotations with the reported value.²⁷⁾ ^f c 2.0, cyclohexane. ^g c 1.0, acetone. ^h Determined by HPLC analysis (column, CHIRALCEL OB, hexane/2-propanol) of 1-phenylethanol or 1-(2-naphthyl)ethanol derived from **8** (LiAlH₄, THF, 0°C, 2h), respectively. ⁱ c 3.0, CHCl₃. ^j c 2.5, CHCl₃. ^k Determined by ¹H-NMR, ¹⁹F NMR or HPLC analysis of the corresponding MTPA ester. ^l c 3.4, CHCl₃. ^m Determined by ¹H NMR in the presence of chiral shift reagent, Eu(hfc)₃. ⁿ c 2.9, CHCl₃. ^o c 1.0, CHCl₃. ^p The reaction was conducted in a mixture of dry diisopropyl ether (10 mL) and dry toluene (10 mL) by the use of **2a** (1.0 equiv. of **7e**).

Kinetic resolution of racemic **7b-e** using isopropenyl acetate (**2a**) proceeded more slowly than that of **7a**, but afforded the product esters **8b-e** in high optical yield (over 93 % e.e.) and alcohols **7b-e** with 80-94 % e.e. The absolute configurations of esters **8b-d**, and **8g** were established by reduction to the corresponding 1-arylethanol with LiAlH_4 (Scheme I-3) followed by comparison of optical rotations with the reported ones.^{26, 36, 37, 38} The reduction of **8c** resulted in the loss of the Br atom on the aromatic ring to give 1-phenylethanol (**9a**).



Scheme I-3 Stereochemical Correlation of **8b-d** and **g**

Optical purities of **8c-e** were determined by $^1\text{H-NMR}$ spectroscopy in the presence of a chiral shift reagent, $\text{Eu}(\text{hfc})_3$. Those of **8b** and **8g** were determined by HPLC analysis of the corresponding 1-arylethanol **9b** (from **8b**) and **9a** (from **8g**) using a column with chiral stationary phase.

The absolute configuration of **8e** was determined by $^1\text{H-NMR}$ as follows. In the presence of a chiral shift reagent, $\text{Eu}(\text{hfc})_3$, $^1\text{H-NMR}$ of (+)-**8e** showed a pair of base-line separated signals assignable to the acetyl protons. The integration of the two signals were not equal, but larger for the peak in the lower magnetic field. This was also true for the enantiomers (*S*)-(+)-**8a**, **c** and **d**. With this correlation, the absolute configuration of (+)-**8e** was determined as *S*.

In summary, enol esters **2** were the most effective acylating reagents for the lipase-catalyzed transesterification in organic solvent. By using this

enzymatic reaction system, the kinetic resolution of racemic 2-halo-1-arylethanol **7a-e** was successfully accomplished with nearly complete stereoselection. Considering the broad substrate specificity of the lipase, this system will provide a versatile and useful method for preparation of optically active alcohols.

I-3 EXPERIMENTAL

General Procedure.

The following instrumentation is relevant to all the experimental sections of this thesis. $^1\text{H-NMR}$ spectra were measured in CDCl_3 with TMS as an internal standard at 60 MHz on a Varian EM-360 spectrometer or at 200 MHz on a Varian VXR-200 spectrometer. $^{13}\text{C-NMR}$ (50 MHz) and $^{19}\text{F-NMR}$ (188 MHz) were measured on a VXR-200 spectrometer. Infrared spectra were recorded on a Hitachi 215 spectrometer. Mass spectra were obtained on a JEOL JMX-DX-300 spectrometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Elemental analyses were performed on a Yanako MT-3 apparatus. Melting points are uncorrected. HPLC analyses were carried out on a Jasco BIP-1 using a silica gel column (NUCLEOSIL 50-5, 4 mm ϕ \times 250 mm, Chemco Pak, hexane : AcOEt = 10 : 1 to 20 : 1, 1 mL/min, detected at 254 nm) or an ODS column (CHEMCOSORB 10-ODS-H, 4.6 mm ϕ \times 250 mm, Chemco Pak; CH_3CN (8) : H_2O (2), 0.5 mL/min, detected at 280 nm). For the analyses of enantiomer, columns with chiral stationary phase (CHRAL CEL OB, 4.6 mm ϕ \times 250 mm, hexane (9) : 2-propanol (1), 0.5 mL/min, detected at 254 nm) and (AK-03, 4.6 \times 250 mm, YMC-Pack; hexane (70) : CH_2Cl_2 (30) : EtOH (2), 1 mL/min, detected at 235 nm) were used. GC analyses were performed on a Shimadzu GC-4B equipped with a packed column (2 % XE-60 on CHROMOSORB-W, 2 m) or on a Shimadzu GC-14A equipped with a capillary column (DB-5, 0.25 μm thick, 0.25 mm ϕ \times 30 m). Data processing of chromatograms were performed on a Hitachi M-833 Chromato-Processor and Jasco DS-300 Data Station for HPLC. The products were isolated by flash-column chromatography on silica gel or on a precoated silica gel glass plate [Kieselgel 60, Merck Co., Ltd.] and bulb-to-bulb distillation on a Büchi Kugelrohr apparatus. Diisopropyl ether, dimethoxyethane (DME), and toluene were distilled over CaH_2 and stored

over 4Å molecular sieves. Dry tetrahydrofuran (THF) was prepared by distillation from benzophenone ketyl and used immediately. Enol esters were all commercially available (Tokyo Chemical Industry Co., Ltd.) and purified by distillation before use. The purity of them was ascertained by GLC and $^1\text{H-NMR}$. 2,2,2-Trichloroethyl acetate was prepared by mixing 2,2,2-trichloroethanol and an equimolar amount of acetic anhydride in the presence of concentrated H_2SO_4 (room temperature, 12 h), and purified by distillation [bp. (bath temp.) 62–72 °C / 14 mmHg, 91 % yield; $^1\text{H-NMR}$ (60 MHz) δ 2.24 (s, 3H, OAc), 4.85 (s, 2H, CH_2)].

The lipase from *Pseudomonas* sp. M-12-33 (trade name, Lipase P Amano) was purchased from Amano Pharmaceutical Co., Ltd. The powder received was dried in a desiccator over P_2O_5 under reduced pressure (room temperature, more than 3 days).

Lipase-Catalyzed Irreversible Acylation of 1-Hexanol Using Enol Esters as Acylating reagent.

Reaction of 1-Hexanol (1) with Isopropenyl Acetate (2a); Typical Procedure.

1-Hexanol (1) (1.02 g, 10.0 mmol) was dissolved in dry diisopropyl ether (20 mL) containing ethyl benzene (0.4 mL) as an internal standard. Lipase from *Pseudomonas* sp. M-12-33 (500 mg) was suspended to the solution and the mixture was stirred for 20 min at 25 °C. The reaction was initiated by adding isopropenyl acetate (2a) (1.05 g, 10.5 mmol), and the mixture was stirred at 25 \pm 1 °C. The proceeding of reaction was monitored by GLC (2 % XE-60 on Chromosorb-W, 2 m, 70 °C, carrier gas N_2 40 mL/min). The retention times were 1.6 min for ethylbenzene, 3.3 min for 1-hexanol, and 4.4 min for hexyl acetate. The conversion of the reaction was calculated from the decrease of 1-hexanol compared with the internal standard.

Preparation of 2-Halo-1-arylethanol 7a-e

(\pm)-2-Chloro-1-phenylethanol (7a); Typical Procedure.

To a stirred solution of 2-chloro-1-phenylethanone (6a) (15.5 g, 100 mmol) in methanol (50 mL), was added sodium borohydride (NaBH_4) (1.90 g, 50 mmol) portionwise to maintain the temperature of the solution

below 0 °C. The mixture was stirred at 0 °C for 30 min and then at room temperature for further 30 min. The reaction was quenched with 2N HCl (50 mL) at 0 °C, and methanol was removed by evaporation. The resulting aqueous solution was extracted with CH_2Cl_2 (3 \times 50 mL) and the combined extracts were washed with brine (3 \times 30 mL) and dried (Na_2SO_4). The solvent was removed *in vacuo* and the residue was distilled to afford 7a as a colorless oil: 14.07 g (90 % yield); bp. (bath temp.) 105–115 °C / 3 mmHg; $^1\text{H-NMR}$ (200 MHz) δ 2.15 (br s, 1H, OH), 3.63 (dd, 1H, J = 11.2 and 8.4 Hz, CH_2), 3.73 (dd, 1H, J = 11.2 and 3.6 Hz, CH_2), 4.88 (dd, 1H, J = 8.4 and 3.6 Hz, CH), and 7.30–7.55 (m, 5 H_{arom}); $^{13}\text{C-NMR}$ (50 MHz) δ 50.81, 74.07, 126.09, 128.46, 128.67, and 139.98.

Compounds 7b–d were prepared by the same procedure from the corresponding ketones 6b–d. Only the purification method, physical state, yield, and $^1\text{H-NMR}$ data are given.

(\pm)-2-Bromo-1-(2-naphthyl)ethanol (7b).

NaBH_4 -reduction of 2-bromo-1-(2-naphthyl)ethanone (6b) followed by crystallization from Et_2O / light petroleum gave a colorless powder: 2.56 g (85 % yield); mp. 65–66 °C; $^1\text{H-NMR}$ (200 MHz) δ 2.72 (br s, 1H, OH), 3.62 (dd, 1H, J = 10.4 and 8.7 Hz, CH_2), 3.73 (dd, 1H, J = 10.4 and 3.5 Hz, CH_2), 5.10 (dd, 1H, J = 8.7 and 3.4 Hz, CH), 7.42–7.58 and 7.78–7.91 (m, 7 H_{arom}).

(\pm)-2-Bromo-1-(4-bromophenyl)ethanol (7c).

NaBH_4 -reduction of 2-bromo-1-(4-bromophenyl)ethanone (6c) followed by distillation gave a colorless oil: 4.48 g (89 % yield); bp. (bath temp.) 122–130 °C / 0.2 mmHg; $^1\text{H-NMR}$ (200 MHz) δ 2.73 (br s, 1H, OH), 3.48 (dd, 1H, J = 10.5 and 8.4 Hz, CH_2), 3.60 (dd, 1H, J = 10.5 and 3.6 Hz, CH_2), 4.88 (dd, 1H, J = 3.6 and 8.4 Hz, CH), 7.25 (d, 2H, J = 8.8 Hz, 2',6'- H_{arom}), and 7.50 (d, 2H, J = 8.8 Hz, 3',5'- H_{arom}).

(\pm)-2-Bromo-1-(4-methoxyphenyl)ethanol (7d).

NaBH_4 -reduction of 2-bromo-1-(4-methoxyphenyl)ethanone (6d) followed by flash column chromatography on silica gel [hexane (4) : AcOEt (1)] gave a colorless oil: 7.05 g (70 % yield); $^1\text{H-NMR}$ (200 MHz) δ 2.40 (br s, 1H, OH), 3.51 (dd, 1H, J = 10.6 and 8.4 Hz, CH_2), 3.60 (dd, 1H, J = 10.6 and 4.0 Hz, CH_2), 3.80 (s, 3H, OMe), 4.88 (dd, 1H, J = 8.4 and 4.0 Hz,

CH), 6.89 (d, 2H, $J = 8.8$ Hz, 2',6'-H_{arom}), and 7.30 (d, 2H, $J = 8.8$ Hz, 3',5'-H_{arom}).

(±)-2-Chloro-1-(3,4-dimethoxyphenyl)ethanol (7e).

A mixture of 2-chloro-1-(3,4-dihydroxyphenyl)ethanone (3.00 g, 16.1 mmol), dimethyl sulfate (6.76 g, 53.6 mmol), and anhydrous K₂CO₃ powder (4.44 g, 32.2 mmol) in dry acetone (40 mL) was refluxed for 7 h under an argon atmosphere. The reaction mixture was filtered over Celite 545 and the filtrate was evaporated. The residue was diluted with AcOEt (50 mL) and washed with 2N HCl (40 mL), sat. NaHCO₃ (40 mL), and brine (40 mL) and then dried (Na₂SO₄). The solvent was removed under reduced pressure and the residue was dissolved in methanol and then diluted with Et₂O to afford 2-chloro-1-(3,4-dimethoxyphenyl)ethanone (**6e**) as amorphous powder: 1.79 g (52.0 % yield); ¹H-NMR (200 MHz) δ 3.94 (s, 3H, OMe), 3.96 (s, 3H, OMe), 4.66 (s, 2H, CH₂Br), 6.90 (d, 1H, $J = 8.2$ Hz, 5'-H_{arom}), 7.53 (d, 1H, $J = 2.0$ Hz, 2'-H_{arom}), 7.56 (dd, 1H, $J = 8.2$ and 2.0 Hz, 6'-H_{arom}).

According to the typical procedure described above, **6e** was reduced with NaBH₄ to give (±)-**7e** quantitatively as a colorless oil. The purity was ascertained by TLC and ¹H-NMR and used it without further purification: 1.76 g; ¹H-NMR (200 MHz) δ 2.64 (d, 1H, $J = 3.0$ Hz, OH), 3.62 (dd, 1H, $J = 11.2$ and 8.4 Hz, CH₂), 3.72 (dd, 1H, $J = 11.2$ and 3.8 Hz, CH₂), 3.87 (s, 3H, OMe), 3.89 (s, 3H, OMe), 4.84 (m, 1H, CH), 6.80-6.95 (m, 3H_{arom}).

Lipase-Catalyzed Kinetic Resolution of Racemic 2-Halo-1-arylethanol 7a-e.

Reaction of (±)-2-Chloro-1-phenylethanol (7a) with Isopropenyl Acetate (2a); Typical procedure.

2-Chloro-1-phenylethanol (**7a**) (2.00 g, 12.8 mmol) was dissolved in dry diisopropyl ether (64 mL). Lipase from *Pseudomonas* sp. M-12-33 (6.4 g) and isopropenyl acetate (**2a**) (2.56 g, 25.5 mmol) were added successively to the solution, and the mixture was stirred at room temperature with monitoring the conversion by HPLC [hexane (10) : AcOEt (1)]. The reaction ceased at 52 % conversion (17 h). The lipase powder was removed by filtration and the filtrate was evaporated to give a colorless oil. The ester **8a** and the unreacted alcohol **7a** were separated by column

chromatography on silica gel [hexane/AcOEt, 20:1-15:1] to give optically active **8a** (1.23 g, 52 % yield) and **7a** (0.88 g, 44 % yield).

Compounds (*R*)-**7a-e** and (*S*)-**8a-g** were obtained by this procedure. Satisfactory combustion analyses (± 0.3 % of calculated values) for carbon and hydrogen were obtained for all products.

(R)-(-)-2-Chloro-1-phenylethanol (7a).

$[\alpha]_D^{25} = -51.5^\circ$ (c 2.032, cyclohexane) [lit.²⁷] $[\alpha]_D^{25} = +53.3^\circ$ (c 2, cyclohexane) for optically pure (*S*)-isomer], 97 % e.e.; MS (70 eV) m/e (relative intensity %) 156 (M⁺, 2.7).

(R)-(-)-2-Bromo-1-(2-naphthyl)ethanol (7b).

$[\alpha]_D^{25} = -38.8^\circ$ (c 2.538, CHCl₃). The e.e. was determined by the ¹H-NMR spectroscopy of the corresponding MTPA ester and found to be 80 %.

(R)-(-)-2-Bromo-1-(4-bromophenyl)ethanol (7c).

$[\alpha]_D^{25} = -31.0^\circ$ (c 2.848, CHCl₃). The e.e. was calculated to be 94 % by the HPLC analysis of the corresponding MTPA ester.

(R)-(-)-2-Bromo-1-(4-methoxyphenyl)ethanol (7d).

$[\alpha]_D^{25} = -37.7^\circ$ (c 1.00, CHCl₃); MS (70 eV) m/e (relative intensity %) 230 (M⁺, 8) and 232 ([M+2]⁺, 8). The e.e. was calculated to be 87 % by the ¹⁹F-NMR spectroscopy of the corresponding MTPA ester.

(R)-(-)-2-Chloro-1-(3,4-dimethoxyphenyl)ethanol (7e).

$[\alpha]_D^{25} = -43.1^\circ$ (c 1.04, CHCl₃). The e.e. was calculated to be 87 % by the ¹H-NMR spectroscopy of the corresponding MTPA ester.

(S)-(+)-2-Chloro-1-phenylethyl Acetate (8a).

Prepared from racemic **7a** and isopropenyl acetate (**2a**), $[\alpha]_D^{25} = +73.2^\circ$ (c 2.020, acetone) [lit.²⁷] $[\alpha]_D^{25} = -80.0^\circ$ (c 2, acetone) for optically pure (*R*)-isomer], 92 % e.e.; ¹H-NMR (200 MHz) δ 2.14 (s, 3H, OAc), 3.71 (dd,

1H, $J = 11.6$ and 4.8 Hz, CH₂), 3.80 (dd, 1H, $J = 11.6$ and 7.7 Hz, CH₂), 5.96 (dd, 1H, $J = 7.7$ and 4.8 Hz, CH), and 7.22-7.47 (m, 5H_{arom}).

(S)-(+)-2-Bromo-1-(2-naphthyl)ethyl Acetate (8b).

Prepared from racemic **7b** and isopropenyl acetate (**2a**), $[\alpha]_{\text{D}}^{25} = +70.0^\circ$ (c 3.026, CHCl₃); ¹H-NMR (200 MHz) δ 2.17 (s, 3H, OAc), 3.66 (dd, 1H, $J = 11.0$ and 5.0 Hz, CH₂), 3.75 (dd, 1H, $J = 11.0$ and 7.8 Hz, CH₂), 6.14 (dd, 1H, $J = 7.8$ and 5.0 Hz, CH), 7.40-7.55 and 7.75-8.00 (m, 7H_{arom}). The e.e. was determined as 95 % by an HPLC analysis of the corresponding (*R*)-(+)-1-(2-naphthyl)ethanol prepared by LiAlH₄-reduction of **8b** (*vide infra*).

(S)-(+)-2-Bromo-1-(4-bromophenyl)ethyl Acetate (8c).

Prepared from racemic **7c** and isopropenyl acetate (**2a**), $[\alpha]_{\text{D}}^{25} = +56.6^\circ$ (c 3.394, CHCl₃); ¹H-NMR (200 MHz) δ 2.14 (s, 3H, OAc), 3.54 (dd, 1H, $J = 10.8$ and 5.4 Hz, CH₂), 3.62 (dd, 1H, $J = 10.8$ and 7.4 Hz, CH₂), 5.91 (dd, 1H, $J = 7.4$ and 5.4 Hz, CH), 7.22 (d, 2H, $J = 8.9$ Hz, 2',6'-H_{arom}), and 7.50 (d, 2H, $J = 8.9$ Hz, 3',5'-H_{arom}). MS (70 eV) m/e (relative intensity %) 320 (M⁺, 1.2) and 322 ([M+2]⁺, 2). The e.e. was found to be 95 % by ¹H-NMR in the presence of a chiral shift reagent, Eu(hfc)₃, [ca. 5 mg of Eu(hfc)₃ for 10 mg of **8c** in 700 μ L of CDCl₃; δ (OAc) 2.68 for (*R*) and 2.73 for (*S*)].

(S)-(+)-2-Bromo-1-(4-methoxyphenyl)ethyl Acetate (8d).

Prepared from racemic **7d** and isopropenyl acetate (**2a**), $[\alpha]_{\text{D}}^{25} = +73.4^\circ$ (c 1.03, CHCl₃); ¹H-NMR (200 MHz) δ 2.11 (s, 3H, OAc), 3.54 (dd, 1H, $J = 10.8$ and 5.0 Hz, CH₂), 3.65 (dd, 1H, $J = 10.8$ and 8.2 Hz, CH₂), 3.80 (s, 3H, OMe), 5.92 (dd, 1H, $J = 8.2$ and 5.0 Hz, CH), 6.89 (d, 2H, $J = 8.9$ Hz, 2',6'-H_{arom}), and 7.28 (d, 2H, $J = 8.9$ Hz, 3',5'-H_{arom}); MS (70 eV) m/e (relative intensity %), 272 (M⁺, 7) and 274 ([M+2]⁺, 7). The e.e. was determined as 93 % [¹H-NMR using Eu(hfc)₃; δ (OAc) 2.51 for (*R*) and 2.54 for (*S*)].

(S)-(+)-2-Chloro-1-(3,4-dimethoxyphenyl)ethyl Acetate (8e).

Prepared from racemic **7e** and isopropenyl acetate (**2a**), $[\alpha]_{\text{D}}^{25} = +83.2^\circ$ (c 1.02, CHCl₃); ¹H-NMR (200 MHz) δ 2.12 (s, 3H, OAc), 3.68 (dd, 1H, $J =$

11.6 and 4.8 Hz, CH₂), 3.79 (dd, 1H, $J = 11.6$ and 7.9 Hz, CH₂), 3.87 (s, 3H, OMe), 3.89 (s, 3H, OMe), 5.89 (dd, 1H, $J = 7.9$ and 4.8 Hz, CH), and 6.80-6.96 (m, 3H_{arom}). The e.e. was calculated to be 97 % [¹H-NMR, Eu(hfc)₃; δ (OAc) 2.16 for (*R*) and 2.18 for (*S*)].

(S)-(+)-2-Chloro-1-phenylethyl Butyrate (8f).

Prepared from racemic **7a** and vinyl butyrate (**2c**), $[\alpha]_{\text{D}}^{25} = +66.2^\circ$ (c 1.02, acetone) [lit.²⁷] $[\alpha]_{\text{D}}^{25} = -68.6^\circ$ (c 1, acetone) for optically pure (*R*)-isomer], 97 % e.e.; ¹H-NMR (200 MHz) δ 0.94 (2 \times t, 3H, $J = 7.4$ Hz, CH₃), 1.68 (sextet, 2H, $J = 7.4$ Hz, CH₂CH₃), 2.38 (t, 2H, $J = 7.4$ Hz, COCH₂), 3.71 (dd, 1H, $J = 11.6$ and 4.9 Hz, CH₂), 3.79 (dd, 1H, $J = 11.6$ and 7.6 Hz, CH₂), 5.97 (dd, 1H, $J = 7.6$ and 4.9 Hz, CH), and 7.30-7.40 (m, 5H_{arom}).

(S)-(+)-2-Chloro-1-phenylethyl Octanoate (8g).

Prepared from racemic **7a** and vinyl octanoate (**2d**), $[\alpha]_{\text{D}}^{25} = +47.9^\circ$ (c 1.01, acetone) [lit.²⁷] $[\alpha]_{\text{D}}^{25} = -46.3^\circ$ (c 1, acetone) for (*R*)-isomer]. The e.e. was calculated to be 96 % by comparing the optical rotation value with the one derived from the LiAlH₄-reduction experiment (*vide infra*); ¹H-NMR (200 MHz) δ 0.86 (m, 3H, CH₃), 1.12-1.40 (m, 8H, (CH₂)₄), 1.64 (m, 2H, COCH₂CH₂), 2.39 (2 \times t, 2H, $J = 7.4$ Hz, COCH₂), 3.71 (dd, 1H, $J = 11.6$ and 4.8 Hz, CH₂), 3.79 (dd, 1H, $J = 11.6$ and 7.6 Hz, CH₂), 5.96 (dd, 1H, $J = 7.6$ and 4.8 Hz, CH), and 7.30-7.40 (m, 5H_{arom}).

Stereochemical Correlation of Enzymatically Prepared Esters; LiAlH₄-Reduction of Esters 8b-d and g.

Stereochemical Correlation of (+)-8b; Typical Procedure.

Ester **8b** [100 mg, 0.34 mmol, $[\alpha]_{\text{D}}^{25} = +70.0^\circ$ (c 3.026, CHCl₃)] was reduced with LiAlH₄ (25.8 mg, 0.68 mmol) at 0 °C in dry THF (5 mL) for 3 h. The usual work-up and chromatographic purification gave (*R*)-(+)-1-(2-naphthyl)ethanol (**9b**): 25.7 mg (44 % yield); $[\alpha]_{\text{D}}^{25} = +33.7^\circ$ (c 1.29, EtOH) [lit.^{36, 37}] $[\alpha]_{\text{D}}^{25} = +41.3^\circ$ (c 5.07, EtOH) for (*R*)-isomer]; ¹H-NMR (200 MHz) δ 1.58 (d, 3H, $J = 6.4$ Hz, Me), 1.92 (br s, 1H, OH), 5.07 (q,

^1H , $J = 6.4$ Hz, CH), 7.23-7.53 and 7.75-7.88 (m, 7H_{arom}). The e.e. was calculated to be 95 % by HPLC (CHIRALCEL OB, hexane (9) : 2-propanol (1), 0.3 mL/min, detected at 280 nm, $R_t = 43.2$ min for (*S*) and 47.7 min for (*R*), $\alpha = 1.15$).

Stereochemical Correlation of (+)-8c.

Ester **8c** with $[\alpha]_{\text{D}}^{25} = +56.6^\circ$ (c 3.394, CHCl_3) was reduced with LiAlH_4 (DME, reflux 12 h) to give (*R*)-(+)-1-phenylethanol (**9a**). $[\alpha]_{\text{D}}^{25} = +51.4^\circ$ (c 1.56, CHCl_3) [lit.³⁹] $[\alpha]_{\text{D}}^{25} = -50.2^\circ$ (c 5.11, CHCl_3) for (*S*)-isomer (93 % e.e.); ^1H -NMR (200 MHz) δ 1.50 (d, 3H, $J = 6.4$ Hz, Me), 1.84 (s, 1H, OH), 4.89 (q, 1H, $J = 6.4$ Hz, CH), 7.20-7.45 (m, 5H_{arom}). The e.e. was determined as 96 % by HPLC (CHIRALCEL OB, hexane (9) : 2-propanol (1), 0.5 mL/min, detected at 254 nm, $R_t = 14.9$ min for (*S*) and 18.3 min for (*R*), $\alpha = 1.50$).

Stereochemical Correlation of (+)-8d.

Ester **8d** with $[\alpha]_{\text{D}}^{25} = +73.4^\circ$ (c 1.03, CHCl_3) was reduced with LiAlH_4 (THF, 0 °C, 3h) to give (*R*)-(+)-1-(4-methoxyphenyl)ethanol (**9d**) $[\alpha]_{\text{D}}^{25} = +31.1^\circ$ (c 2.54, EtOH) [lit.³⁸] $[\alpha]_{\text{D}}^{20} = +19.4^\circ$ (EtOH) for partially resolved (*R*)-isomer; ^1H -NMR (200 MHz) δ 1.47 (d, 3H, $J = 6.5$ Hz, Me), 1.83 (br s, 1H, OH), 3.79 (s, 3H, OMe), 4.84 (q, 1H, $J = 6.5$ Hz, CH), 6.87 (d, 2H, $J = 9.0$ Hz, 2',6'- H_{arom}) and 7.29 (d, 2H, $J = 9.0$ Hz, 3',5'- H_{arom}). The e.e. was determined as 84 % by HPLC (CHIRALCEL OB, hexane (9) : 2-propanol (1), 0.5 mL/min, detected at 254 nm, $R_t = 30.0$ min for (*S*) and 36.7 min for (*R*), $\alpha = 1.32$).

LiAlH_4 -Reduction of (*S*)-(+)-8g.

Ester **8g** with $[\alpha]_{\text{D}}^{25} = +45.7^\circ$ (c 1.83, acetone) was reduced with LiAlH_4 (THF, 0 °C, 3h). After the usual work-up, the resulted ether solution of 1-phenylethanol (**9a**) was afforded. A small portion of the ethereal solution was directly analyzed by HPLC (CHIRALCEL OB, hexane (9) : 2-propanol (1), 0.5 mL/min, detected at 254 nm, $R_t = 14.8$ min for (*S*) and 18.4 min for (*R*), $\alpha = 1.46$). The e.e. was calculated to be 92 %.

Preparation of Diastereomeric MTPA Ester of Resolved Alcohols.

MTPA Ester of (-)-7e; Typical Procedure:

A mixture of (-)-**7e** (10.0 mg, 0.046 mmol), dry pyridine (122.3 mg, 1.55 mmol), and (*R*)- α -methoxy- α -(trifluoromethyl)phenylacetyl chloride (50.0 mg, 0.20 mmol) in dry benzene (2 mL) was stirred overnight at room temperature under an argon atmosphere. After **7e** was completely consumed [TLC, hexane (4) : AcOEt (1)], the reaction mixture was treated with 2N HCl (10 mL) and extracted with ether (2×5 mL). The combined extracts were successively washed with sat. NaHCO_3 (10 mL) and brine (10 mL), and then dried (Na_2SO_4). The solvent was removed in vacuo and the product mixture was analyzed by ^1H -NMR. The e.e. was calculated to be 87 % from the base-line separated signals of methyn proton: $[\delta$ 6.04 (dd, $J = 9.6$ and 3.8 Hz) and 6.13 (dd, $J = 8.6$ and 4.2 Hz), their integral ratio was 9.4 : 103.3].

MTPA ester of (-)-7b:

From the ^1H -NMR signals of base-line separated methyn protons of diastereomers: $[\delta$ 6.29 (dd, $J = 9.2$ and 3.6 Hz) and 6.39 (dd, $J = 8.4$ and 4.8 Hz), their integral ratio was 8.4 : 77.3]. The e.e. was calculated to be 80 %.

MTPA ester of (-)-7c:

Although the ^1H -NMR signals of the methyn proton were not separated incompletely, the HPLC analysis [NUCLEOSIL 50-5, hexane (10) : AcOEt (1), 0.7 mL/min, detected at 254 nm, $R_t = 9.5$ min for (*S*) and 10.1 min for (*R*), $\alpha = 1.17$] succeeded in separation of the two diastereomers. The e.e. was determined as 94 %.

MTPA ester of (-)-7d:

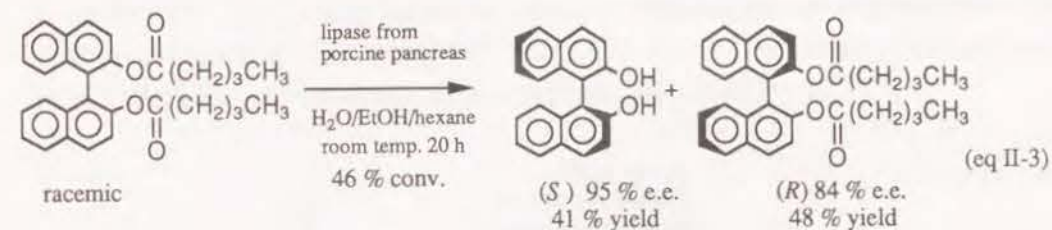
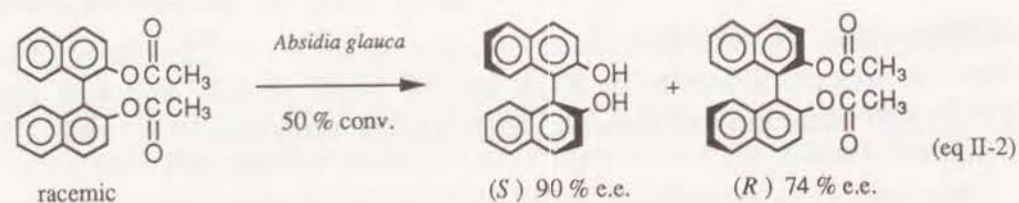
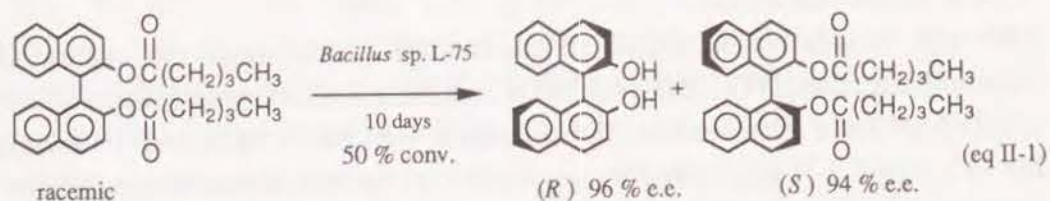
The e.e. was calculated to be 87 % by ^{19}F -NMR $[\delta$ 4.27 and 4.35 from CF_3COOH as an internal standard, their integral ratio was 10.2 : 146.7].

CHAPTER II

Lipase-Catalyzed Stereoselective Acylation of [1,1'-Binaphthyl]-2,2'-diol and Deacylation of Its Esters in Organic Solvent

II-1 INTRODUCTION

Optically active [1,1'-binaphthyl]-2,2'-diol (binaphthol) is recognized as an effective chiral auxiliary of asymmetric reagents^{40, 41)} because of its C_2 axial dissymmetry and molecular flexibility. For example, chiral binaphthol was converted into a chiral catalyst 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (BINAP) for asymmetric hydrogenation⁴²⁾ and chiral crown ethers^{43, 44)} for stereoselective inclusion. Moreover, it served as a chiral auxiliary to prepare chiral hydride reagent (BINAL-Hs).⁴⁵⁾ Recent development in biochemical catalysts for organic synthesis has enabled optically active binaphthol to be prepared from the microbial (eq II-1),⁴⁶⁾ (eq II-2)⁴⁷⁾ and the enzymatic hydrolysis (eq II-3)⁴⁸⁾ of the racemic diesters of binaphthol.



These methods are more convenient routes for preparing optically active binaphthol than the tedious chemical methods developed so far;^{49, 50, 51, 52)} however, these biochemical reactions were carried out in a dilute aqueous solution or in an emulsion since binaphthol and its esters are practically insoluble in aqueous buffers.

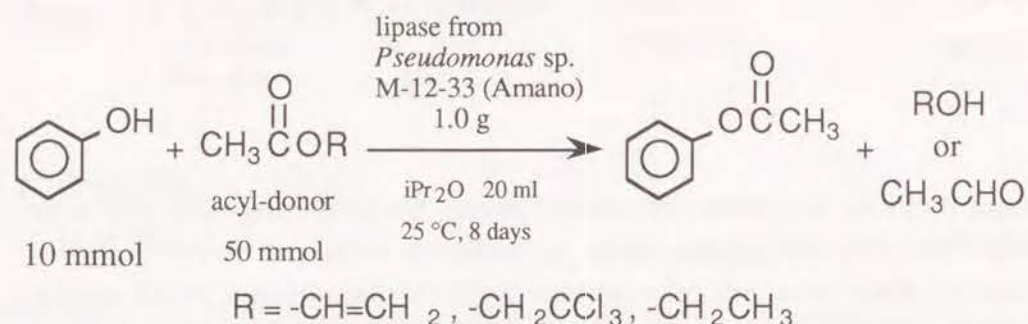
The author designed a kinetic resolution of racemic binaphthol and its esters through the stereoselective acylation and deacylation catalyzed by lipase in organic solvent. First, acylating reagents and lipases were examined for the acylation of the less nucleophilic hydroxyl groups of binaphthol. In the preceding chapter, it was demonstrated that enol esters such as vinyl acetate or isopropenyl acetate irreversibly reacted with aliphatic alcohols in the presence of lipase much more rapidly than trichloroethyl acetate. Enol esters were therefore selected and tested their potential of acylation for phenolic hydroxyl groups.

II-2 RESULTS AND DISCUSSION

Lipase-Catalyzed Acylation of Racemic [1,1'-Binaphthyl]-2,2'-diol Using Enol Esters as Acylating Reagent

As a preliminary experiment, phenol was allowed to react with 5 equivalent moles of vinyl acetate (**1a**) in the presence of lipase from *Pseudomonas* sp. M-12-33 (Amano Pharm. Co., Ltd.) in diisopropyl ether (Scheme II-1). The conversion of the reaction reached 99 % in 8 days, and phenyl acetate was obtained in 98 % chemical yield. Under the same reaction conditions, 2,2,2-trichloroethyl acetate gave only 8 % conversion, and no reaction was observed when ethyl acetate was used (Table II-1).

Thus it was suggested that enol esters could be a good acylating reagent for the hydroxyl groups of binaphthol.



Scheme II-1 Lipase-Catalyzed Acetylation of Phenol

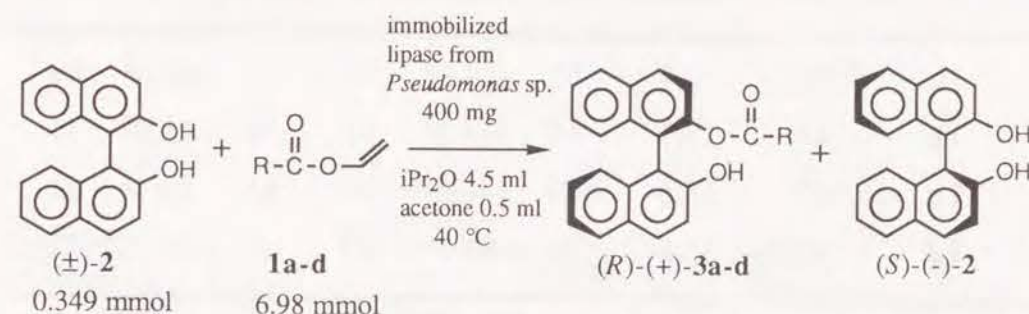
Table II-1 Lipase-Catalyzed Acetylation of Phenol^a

acyl-donor	conversion ^b (%)
vinyl acetate	99 ^c
2,2,2-trichloroethyl acetate	8
ethyl acetate	0

^a Conditions: phenol (0.94 g, 10.0 mmol), acyl-donor (50.0 mmol), lipase (1.0 g) from *Pseudomonas* sp. M-12-33, dry iPr₂O (20 mL), 25 ± 1 °C, 8 days. ^b Determined by GLC (2 % XE-60, 2 m, 120 °C). ^c After the filtration of the lipase powder, the filtrate was evaporated and distilled to afford phenyl acetate: 1.33 g (98 % chemical yield).

A preliminary experiment, however, showed that this lipase from *Pseudomonas* sp. M-12-33 did not catalyze the acylation of binaphthol under the above experiment conditions. Then, commercially available lipase preparations were screened for the catalytic activity of this acylation.

Out of fourteen lipase preparations tested, lipase from *Pseudomonas* sp. (Toyobo Co., Ltd.) was found to catalyze the acylation of binaphthol (**2**) with vinyl acetate (**1a**), vinyl butyrate (**1b**), vinyl hexanoate (**1c**) and vinyl octanoate (**1d**) with a reasonable reaction rate (Scheme II-2).



a: R = CH₃-; b: CH₃(CH₂)₂-; c: CH₃(CH₂)₄-; d: CH₃(CH₂)₆-

Scheme II-2 Lipase-Catalyzed Stereoselective Acylation of (±)-2 Using **1a-d** as Acylating Reagent

Anhydrous diisopropyl ether containing 10 % (v/v) of dry acetone was the solvent of choice, since binaphthol was hardly dissolved in non-polar organic solvent such as hexane, benzene and toluene which were suitable for several lipase-catalyzed reactions.⁸⁾ The reaction was carried out at 40 °C and the conversion of the reaction was monitored by HPLC. The results are summarized in Table II-2.

The diester of binaphthol was not formed under the reaction conditions, in which an excess amount of acylating reagents **1a-d** was used, indicating that the lipase selectively catalyzed the mono-acylation but not di-acylation of binaphthol. The reaction with vinyl acetate (**1a**) ceased at the 52 % conversion after 2.7 days, while the acylations with the other vinyl esters **1b-d** proceeded very slowly. When the optimal conversion was reached, the lipase powder was removed by filtration and monoesters **3** and binaphthol (**2**) were separated by silica gel column chromatography.

Table II-2 Lipase-catalyzed Stereoselective Acylation of (\pm)-**2** Using **1** as Acylating Reagent^a

vinyl ester	con- version ^b (%)	reaction time (day)	<i>(R)</i> -(+)- 3			<i>(S)</i> -(-)- 2		
			yield ^c (%)	$[\alpha]_D^d$ (deg)	e.e. ^e (%)	yield ^c (%)	$[\alpha]_D^d$ (deg)	e.e. ^f (%)
1a	52	2.7	53	+31.0 ^g	95	53	-28.0 ^h	89
1b	45	13.5	46	+41.9 ⁱ	91	54	-25.2 ^j	74
1c	42	13.6	43	+46.0 ^k	91	54	-23.4 ^l	69
1d	43	13.6	46	+45.0 ^m	90	55	-24.7 ⁿ	73

^a Conditions: (\pm)-**2** (100 mg, 0.349 mmol), vinyl ester **1** (6.98 mmol), immobilized lipase powder (400 mg), dry diisopropyl ether (4.5 mL) and dry acetone (0.5 mL), 40 °C.

^b Determined by HPLC (ODS column, CH₃CN (8) : H₂O (2), 0.5 mL/min, 280 nm).

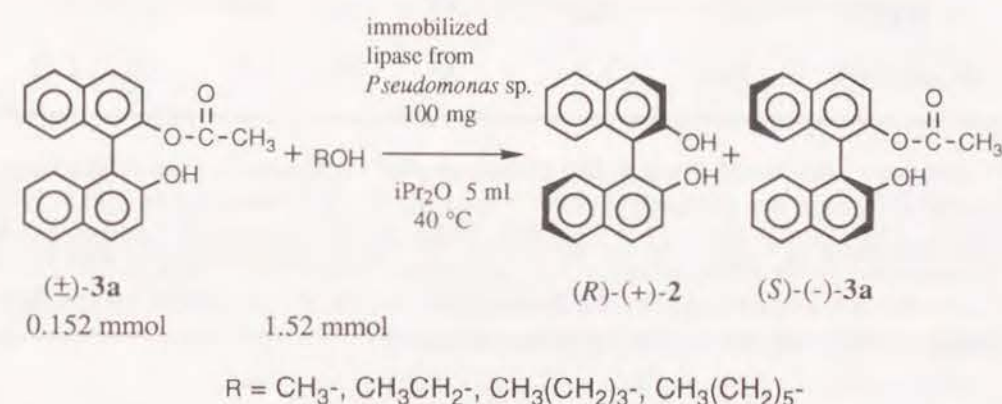
^c Isolated yield based on (\pm)-**2**. ^d Measured in THF at 25 °C. ^e Determined by the HPLC analysis (AK-03 column, hexane (70) : CH₂Cl₂ (30) : EtOH (2), 1 mL/min, 235 nm) of (*R*)-(+)-**2** derived from (+)-**3**. ^f Determined by the HPLC analysis using an AK-03 column. ^g *c* 1.21. ^h *c* 1.05. ⁱ *c* 1.14. ^j *c* 1.10. ^k *c* 1.14. ^l *c* 1.07. ^m *c* 1.31. ⁿ *c* 1.10.

The enantiomeric excesses (e.e.s) of the recovered binaphthol were determined as 69-89 % by HPLC equipped with a column with chiral stationary phase (see experimental). To determine the optical purities of the monoesters **3a-d**, they were hydrolyzed with KOH in methanol to yield binaphthol. From the optical purity of the resulting binaphthol, the e.e.s of the monoesters obtained were calculated and found to be 90-95 %.

The absolute configuration of the recovered binaphthol (-)-**2** was determined as the *S* configuration, based on the (-) sign of the optical rotation.⁵³⁾ On the other hand, the product monoesters (+)-**3** had the *R* configuration, since the hydrolysis of (+)-**3** gave (*R*)-(+)-binaphthol. Consequently, the lipase selectively acylated *R* isomer of binaphthol.

Lipase-Catalyzed Stereoselective Deacylation of Racemic Binaphthyl Monoesters

Another route for the resolution of racemic binaphthol is stereoselective deacylation, alcoholysis, of racemic binaphthyl monoacetate **3a** (Scheme II-3). Racemic **3a** was allowed to react with alcohols such as methanol, ethanol, 1-butanol and 1-hexanol in the presence of the lipase in dry diisopropyl ether. The results are shown in Table II-3.



Scheme II-3 Lipase-Catalyzed Stereoselective Deacylation of (\pm)-**3a** with Alcohols

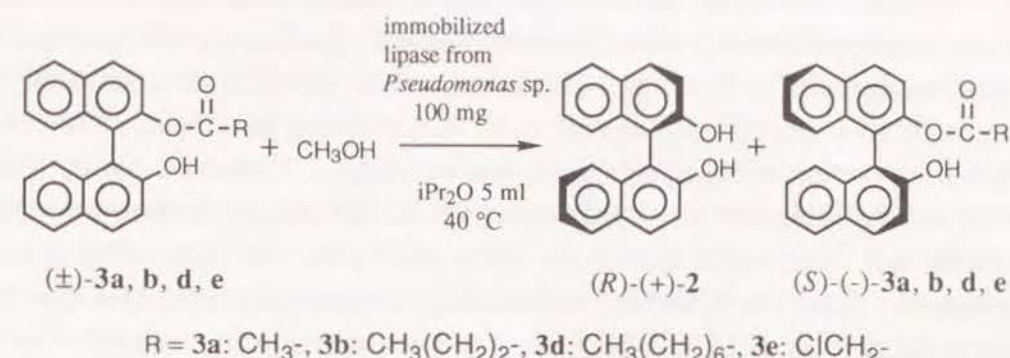
In all entries in Table II-3, the reactions reached 46 % conversion within 4 days to give (*R*)-(+)-binaphthol with 96 % e.e. and (*S*)-(-)-monoacetate with 91-96 % e.e. Under the same reaction conditions, the diacetate of binaphthol was not consumed at all. This result was consistent with the observation that no diester of binaphthol was formed in the acylations of binaphthol. Consequently, the lipase had a strict substrate-specificity for monoacetate **3a**. Enantiomeric ratio (*E* values)⁵⁴⁾ was calculated for each substrate fraction and found to be almost the same. No significant difference was observed in the stereoselectivities and the reaction times among the alcohols used.

Table II-3 Lipase-catalyzed Stereoselective Deacylation of (±)-**3a** with Alcohols^a

alcohol	con- version ^b (%)	reaction time (day)	(S)-(-)- 3a		(R)-(+)- 2		E ^f
			yield ^c (%)	e.e. ^d (%)	yield ^c (%)	e.e. ^e (%)	
CH ₃ OH	46	3.8	44	91	44	96	125
C ₂ H ₅ OH	46	3.8	46	95	36	96	126
nC ₄ H ₉ OH	46	3.8	47	96	44	96	126
nC ₆ H ₁₃ OH	46	3.4	55	95	52	96	121

^a Conditions: (±)-**3a** (50.0 mg, 0.152 mmol), alcohol (1.52 mmol), immobilized lipase powder (100 mg), dry diisopropyl ether (5 mL), 40 °C. ^b Determined by HPLC (ODS column, CH₃CN (8) : H₂O (2), 0.5 mL/min, 280 nm). ^c Isolated yield based on (±)-**3a**. ^d Determined by the HPLC analysis (AK-03 column, hexane (70) : CH₂Cl₂ (30) : EtOH (2), 1 mL/min, 235 nm) of (S)-(-)-**2** derived from (-)-**3a**. ^e Determined by the HPLC analysis using an AK-03 column. ^f Enantiomeric ratio.⁵⁴⁾

Some of the lipase-catalyzed transesterifications in organic solvent were assumed to proceed through an acyl-enzyme intermediate in two successive steps: the acyl-enzyme formation and the deacylation.^{8, 55)} If this assumption holds for the present reaction, the results shown in Table II-3 suggest that, under the present reaction conditions, the acyl-enzyme formation was not only the rate-determining step but also the one where the stereoselectivity was determined. Consequently, the reaction rate should be improved by altering the acyl group of the substrate monoesters. Thus, four different monoesters **3a**, **b**, **d**, and **e** were synthesized and allowed to react with methanol (Scheme II-4). The results are summarized in Table II-4. Monoctanoate **3d** reacted slightly faster than monoacetate **3a** and monobutyrate **3b** with the same stereoselectivity. Increasing in the chain length of the acyl group of the monoesters **3** did little effect to improve the reactivity.

Scheme II-4 Lipase-Catalyzed Methanolysis of (±)-**3a**, **b**, **d**, and **e**Table II-4 Effects of Acyl Groups of Monoesters on the Methanolysis of (±)-**3** Catalyzed by Lipase^a

entry	substrate	con- version ^b (%)	reaction time (h)	(S)-(-)- 3			(R)-(+)- 2		
				yield ^c (%)	[α] _D ^d (deg)	e.e. ^e (%)	yield ^c (%)	[α] _D ^d (deg)	e.e. ^f (%)
1	3a	48	29.4	52	-32.1 ^g	99	55	+28.4 ^h	91
2	3b	49	29.7	50	-44.0 ⁱ	96	47	+31.4 ^j	98
3	3d	51	18.9	46	-48.2 ^k	95	44	+31.0 ^l	98
4 ^m	3e	50	5.2	34	-17.0 ⁿ	99	62	+19.1 ^o	62 ^p

^a Conditions: (±)-**3** (50.0-62.8 mg, 0.152 mmol), methanol (1.52 mmol), immobilized lipase powder (100 mg), dry diisopropyl ether (5 mL), 40 °C. ^b Determined by HPLC (ODS column, CH₃CN (8) : H₂O (2), 0.5 mL/min, 280 nm). ^c Isolated yield based on (±)-**3**. ^d Measured in THF at 25 °C. ^e Determined by the HPLC analysis (AK-03 column, hexane (70) : CH₂Cl₂ (30) : EtOH (2), 1 mL/min, 235 nm) of (S)-(-)-**2** derived from (-)-**3**. ^f Determined by the HPLC analysis using an AK-03 column. ^g c 1.30. ^h c 1.00. ⁱ c 1.37. ^j c 1.01. ^k c 1.44. ^l c 0.97. ^m 2.0 equiv. of methanol were employed as an acyl-acceptor. ⁿ c 0.92. ^o c 1.01. ^p The e.e. of (R)-(+)-**2** was 90 % when the reaction mixture was analyzed by HPLC before the chromatographic separation.

On the other hand, the conversion of monochloroacetate **3e** having an electron-withdrawing chlorine atom at the α -position of the carbonyl group reached 50 % in 5.2 h. The reaction was also found to be highly stereoselective affording (*R*)-(+)-**2** in 90 % e.e. and (*S*)-(-)-**3e** in 99 % e.e. when the reaction mixture was analyzed by HPLC. However, the optical purity of the isolated (*R*)-(+)-**2** was only 62 %. Chloroacetate **3e** was unstable and hydrolyzed readily on silica gel during the chromatographic separation. Thus, the lower e.e. value of the isolated (*R*)-(+)-**2** was due to the contamination of (*S*)-(-)-**2** which was formed non-enzymatically from the chloroacetate (*S*)-(-)-**3e** during the separation process. Although chloroacetate **3e** was not suitable for resolving the binaphthols on a preparative scale because of its instability during the isolation process, this reaction (Scheme II-4) was thus improved by increasing the electrophilicity of the acyl group of the substrates.

In summary, stereoselective acylation of racemic binaphthol was accomplished for the first time by the lipase-catalyzed transesterification with enol esters in organic solvent. The stereoselective deacylation of racemic binaphthyl monoesters was also established. In these two reactions, the lipase showed a strict preference for mono-esters of binaphthol over di-esters. Monoesters **3** and binaphthol (**2**) with high optical purity were obtained in high chemical yields. In deacylation reactions, the reaction rate was improved without changing the stereoselectivity by introducing an electronegative acyl groups to the substrates based on the assumption that the acyl-enzyme formation was the rate-determining step. This idea will be generally applicable to the lipase-catalyzed deacylation of racemic esters.

II-3 EXPERIMENTAL

Preparation of Immobilized Lipase.

The lyophilized powder of lipase from *Pseudomonas* sp. (Toyobo Co., Ltd.) (41.2 mg) was dissolved in 5 mM potassium phosphate buffer (pH 7.0, 22 mL) at 0 °C. Diatomaceous earth (Hyflo Super-Cel, 6.2 g) was added to the enzyme solution. The resulting paste was stirred for 15 min and then spread on a petri dish and dried over CaCl_2 in a desiccator at

room temperature under reduced pressure overnight. After most of the water had been removed, the mixture was further dried over P_2O_5 under reduced pressure for an additional 2 days, giving a lipase preparation adsorbed on Hyflo Super-Cel.

Preparation of Substrate Monoesters **3a**, **b**, **d** and **e**.

(\pm)-2-Acetoxy-2'-hydroxy-1,1'-binaphthyl (**3a**); Typical Procedure.

To a solution of [1,1'-binaphthyl]-2,2'-diol (**2**) (1.00 g, 3.49 mmol) in a mixture of toluene (15 mL), CH_2Cl_2 (10 mL) and pyridine (3 mL), were added 4-(*N,N*-dimethylamino)pyridine (17.1 mg, 0.175 mmol) and acetic anhydride (392 mg, 3.84 mmol) at 0 °C. The mixture was stirred at room temperature for 6 h, and the solvent was removed *in vacuo*. The residue was dissolved in ether (25 mL), and washed with 2N HCl (20 mL), sat. NaHCO_3 (20 mL) and brine (20 mL) successively, and then dried (Na_2SO_4). Evaporation and silica gel column chromatography [hexane (5) : acetone (1)] gave **3a** as an amorphous powder: 1.02 g (89 %); $^1\text{H-NMR}$ δ 1.86 (s, 3H, COCH_3), 5.21 (s, 1H, OH), 7.00-7.05, 7.20-7.55 and 7.82-8.10 (m, 12 H_{arom}); Anal. Calcd. for $\text{C}_{22}\text{H}_{16}\text{O}_3$: C, 80.47; H, 4.91 %. Found: C, 80.34; H, 4.97 %.

(\pm)-2-Butanoyloxy-2'-hydroxy-1,1'-binaphthyl (**3b**).

Prepared from butyric anhydride (276 mg, 1.75 mmol) and binaphthol (500 mg, 1.75 mmol) and followed by silica gel column chromatography [hexane (3) : acetone (1)] gave **3b** as a colorless syrup: 566 mg (91 %); $^1\text{H-NMR}$ δ 0.57 (t, 3H, $J = 7.4$ Hz, CH_3), 1.21 (m, 2H, CH_2CH_3), 2.11 (m, 2H, COCH_2), 5.21 (s, 1H, OH), 7.00-7.08, 7.18-7.55 and 7.80-8.10 (m, 12 H_{arom}); Anal. Calcd. for $\text{C}_{24}\text{H}_{20}\text{O}_3$: C, 80.88; H, 5.66 %. Found: C, 80.77; H, 5.74 %.

(\pm)-2-Octanoyloxy-2'-hydroxy-1,1'-binaphthyl (**3d**).

Prepared from octanoic anhydride (943 mg, 3.49 mmol) and binaphthol (1.00 g, 3.49 mmol) and followed by silica gel column chromatography [hexane (3) : CH_2Cl_2 (2)] gave **3d** as a colorless syrup: 1.28 g (89 %); $^1\text{H-NMR}$ δ 0.81-1.30 (m, 13H, $(\text{CH}_2)_5\text{CH}_3$), 2.12 (m, 2H, COCH_2), 5.22 (s, 1H, OH), 7.02-7.08, 7.21-7.56 and 7.80-8.12 (m, 12 H_{arom}). Anal. Calcd. for $\text{C}_{28}\text{H}_{28}\text{O}_3$: C, 81.52; H, 6.84 %. Found: C, 81.56; H, 7.04 %.

(±)-2-Chloroacetoxy-2'-hydroxy-1,1'-binaphthyl (3e).

To a solution of [1,1'-binaphthyl]-2,2'-diol (1.00 g, 3.49 mmol) and 2,6-dimethylpyridine (449 mg, 4.19 mmol) in dry THF (15 mL), was added dropwisely the THF (2 mL) solution of chloroacetyl chloride (394 mg, 3.49 mmol) room temperature over 20 min, and the mixture was stirred for 1 h. The reaction mixture was evaporated and the residue was dissolved in ether (20 mL). The ethereal solution was washed with 3 % citric acid (10 mL) and brine (20 mL), and dried (Na_2SO_4). Evaporation and silica gel column chromatography [hexane (3) : CH_2Cl_2 (4)] gave **3e** as a colorless syrup: 191 mg (15 %); $^1\text{H-NMR}$ δ 3.70 and 3.82 (2 × d, 2H, J = 14.9 Hz, CH_2Cl), 5.02 (s, 1H, OH), 6.98-7.07, 7.21-7.90 and 7.82-8.15 (m, 12 H_{arom}); MS (70 eV) m/z (relative intensity %) 362 (M^+ , 47), 364 ($[\text{M}+2]^+$, 17), 287 (23), 286 (100), 268 (13), 239 (15) and 115 (8). Found: m/z 362.07315 and 364.06819. Calcd. for $\text{C}_{22}\text{H}_{15}\text{ClO}_3$: M and M+2, 362.07095 and 364.06809; Anal. Calcd. for $\text{C}_{22}\text{H}_{15}\text{ClO}_3$: C, 72.83; H, 4.17 %. Found: C, 72.75; H, 4.73 %.

Lipase-Catalyzed Stereoselective Acylation of Racemic [1,1'-Binaphthyl]-2,2'-diol (2).**Reaction Using Vinyl Acetate (1a) as an Acylating Reagent; Typical Procedure.**

Binaphthol (**2**) (100 mg, 0.349 mmol) and vinyl acetate (**1a**) (601 mg, 6.98 mmol) were dissolved in a mixture of diisopropyl ether (4.5 mL) and acetone (0.5 mL). The immobilized lipase (400 mg) was added to the solution and the resulting suspension was stirred at 40 °C for 66 h. After the lipase powder was removed by filtration, the filtrate was evaporated and the residual oil was chromatographed on silica gel [hexane (3) : CH_2Cl_2 (2)] to give (*R*)-(+)-**3a**: (60.5 mg, 53 % yield) and (*S*)-(-)-**2**: (52.6 mg, 53 % yield).

The conversion of the lipase-catalyzed acylations with **1a-d** was determined by HPLC equipped with an ODS column (CHEMCOSORB 10-ODS-H, 4.6 ϕ × 250 mm, Chemco Pak; CH_3CN (8) : H_2O (2), 0.5 mL/min, detected at 280 nm). The retention times of **2** and **3** were: **2**, 8.0 min; **3a**, 9.0 min; **3b**, 12 min; **3c**, 16 min; and **3d**, 28 min. In monitoring the methanolysis of (±)-**3e**, the flow rate was 0.3 mL/min; the retention times of **2** and **3e** were 13 and 15 min, respectively.

(S)-(-)-[1,1'-Binaphthyl]-2,2'-diol (2).

$[\alpha]_{\text{D}}^{25} = -28.0^\circ$ (c 1.05, THF). (Lit.⁵³) $[\alpha]_{\text{D}}^{25} = +43.0^\circ$ (c 0.9, THF) for the

R isomer). The enantiomeric excess (e.e.) was determined as 89 % by HPLC using a column with chiral stationary phase (AK-03, 4.6 ϕ × 250 mm, YMC-Pack; hexane (70) : CH_2Cl_2 (30) : EtOH (2), 1 mL/min, detected at 235 nm). The retention times of each enantiomer was as follows: (*R*)-(+)-**2**, 12 min; (*S*)-(-)-**2**, 17 min; $\alpha = 1.57$. $^1\text{H-NMR}$ δ 5.05 (s, 2H, OH), 7.11-7.18, 7.25-7.42 and 7.85-8.00 (m, 12 H_{arom}); Anal. Calcd. for $\text{C}_{20}\text{H}_{14}\text{O}_2$: C, 83.90; H, 4.93 %. Found: C, 83.76; H, 5.02 %.

(R)-(+)-2-Acetoxy-2'-hydroxy-1,1'-binaphthyl (3a).

$[\alpha]_{\text{D}}^{25} = +31.0^\circ$ (c 1.21, THF). The e.e. was calculated as 95 % by the

HPLC analysis of the corresponding (*R*)-(+)-**2** derived from (*R*)-(+)-**3a** by alkaline hydrolysis (1.5 mM KOH in methanol, room temperature, the reaction being finished in 2 min).

(R)-(+)-2-Butanoyloxy-2'-hydroxy-1,1'-binaphthyl (3b).

$[\alpha]_{\text{D}}^{25} = +41.9^\circ$ (c 1.14, THF). The e.e. was calculated to be 91 % by the

HPLC analysis of the corresponding (*R*)-(+)-**2** derived from (*R*)-(+)-**3b** as above.

(R)-(+)-2-Hexanoyloxy-2'-hydroxy-1,1'-binaphthyl (3c).

$[\alpha]_{\text{D}}^{25} = +46.0^\circ$ (c 1.14, THF). The e.e. was determined to be 91 % by the

HPLC analysis of the corresponding (*R*)-(+)-**2** derived from (*R*)-(+)-**3c** as above. $^1\text{H-NMR}$ δ 0.72 (m, 3H, CH_3), 0.78-1.80 (m, 6H, $(\text{CH}_2)_3\text{CH}_3$), 2.12 (m, 2H, COCH_2), 5.24 (s, 1H, OH), 7.00-7.08, 7.18-7.55 and 7.80-8.10 (m, 12 H_{arom}); MS (70 eV) m/z (relative intensity %) 384 (M^+ , 20), 287 (46), 286 (100), 268 (12), 239 (12) and 115 (9). Found: m/z 384.17040. Calcd. for $\text{C}_{26}\text{H}_{24}\text{O}_3$: M, 384.17250.

(R)-(+)-2-Octanoyloxy-2'-hydroxy-1,1'-binaphthyl (3d).

$[\alpha]_{\text{D}}^{25} = +45.0^\circ$ (c 1.31, THF). The e.e. was calculated to be 90 % by the

HPLC analysis of the corresponding (*R*)-(+)-**2** derived from (*R*)-(+)-**3d** as above.

Lipase-Catalyzed Stereoselective Deacylation of Racemic Binaphthyl Monoesters 3a, b, d and e.

Methanolysis of (\pm)-3d; Typical Procedure.

The immobilized lipase (100 mg) was suspended in a solution of racemic **3d** (62.8 mg, 0.152 mmol) in diisopropyl ether (5 mL). The reaction was initiated by adding methanol (48.7 mg, 1.52 mmol) and the mixture was stirred at 40 °C for 19 h. After filtration of the lipase powder, the filtrate was evaporated and chromatographed on silica gel [hexane (3) : CH₂Cl₂ (2)] to afford (*R*)-(+)-**2** (19.4 mg, 44 % yield) and (*S*)-(-)-**3d** (28.7 mg, 46 % yield).

(*S*)-(-)-2-Chloroacetoxy-2'-hydroxy-1,1'-binaphthyl (**3e**).

$[\alpha]_D^{25} = -17.0^\circ$ (*c* 0.92, THF). The e.e. was determined as 99 % by the

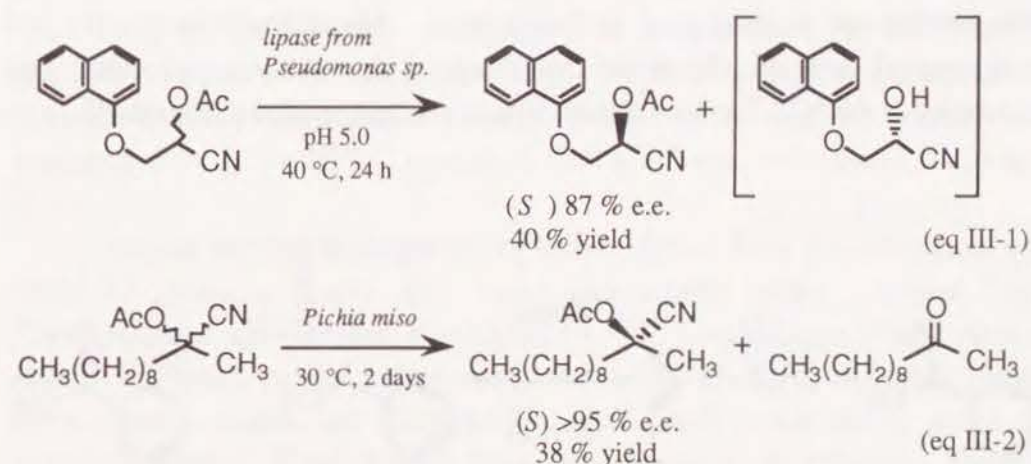
HPLC analysis of the corresponding (*S*)-(-)-**2** derived from (*S*)-(-)-**3e**.

CHAPTER III

Kinetic Resolution of Racemic 1-Cyano-1-phenylmethanol via Stereoselective Acetylation Catalyzed by Lipase in Organic Solvent

III-1 INTRODUCTION

Optically active cyanohydrins are important starting materials for the synthesis of drugs and pesticides because cyanohydrins are easily converted into a variety of chiral synthons such as β -aminoalcohols,^{56, 57, 58)} α -hydroxyacids,⁵⁹⁾ and α -hydroxyketones.⁶⁰⁾ Biochemical approaches to the resolution of racemic cyanohydrins were attained in aqueous buffer by the stereoselective hydrolysis of the corresponding acetates. Matsuo et al.⁵⁶⁾ resolved a racemic cyanohydrin acetate, an intermediate for β -blocker synthesis, using lipase from *Pseudomonas* sp. (eq III-1). Microbial hydrolysis of a racemic ketone cyanohydrin acetate was also conducted by Ohta et al.⁶¹⁾ (eq III-2).



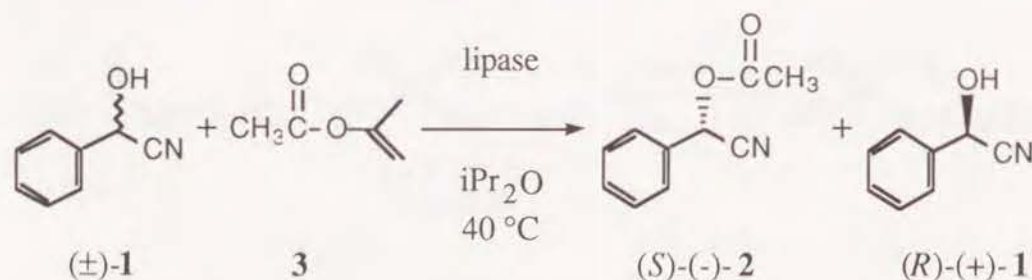
The major disadvantage of these reactions conducted in aqueous medium is that the hydrolyzed product, cyanohydrins, are unstable in

aqueous system and subject to decomposition or racemization, while the unreacted esters are recovered in good optical yield.^{62, 63} In the reaction (eq III-2), for example, the cyanohydrin, the hydrolyzed product, was decomposed to the corresponding ketones and hydrogen cyanide. One of the solutions to this drawback is to use organic solvent because cyanohydrins are more stable in organic solvent than in aqueous medium. In this chapter, the kinetic resolution of racemic 1-cyano-1-phenylmethanol (mandelonitrile) was performed in organic solvent through the stereoselective acylation catalyzed by lipase. Since mandelonitrile is an alcohol having an electron-withdrawing cyano group and a bulky phenyl group on the α -position, the reactivity as a nucleophile is thought to be lower than primary aliphatic alcohols. Therefore a powerful acylating reagent, an enol ester is required to accelerate the reaction.

III-2 RESULTS AND DISCUSSION

Lipase-Catalyzed Stereoselective Acetylation of Racemic 1-Cyano-1-phenylmethanol

Six commercially available preparations of lipase were tested for the acetylation of racemic 1-cyano-1-phenylmethanol (mandelonitrile) (\pm)-(1). The results are summarized in Table III-1. Mandelonitrile (\pm)-(1) and isopropenyl acetate (3) were dissolved in dry diisopropyl ether and incubated at 40 °C. To this solution lipase was suspended (Scheme III-1).



Scheme III-1 Lipase-Catalyzed Stereoselective Acetylation of (\pm)-1

Table III-1 Screening of the Lipase Preparations for Stereoselective Acetylation of Mandelonitrile (1)^a

origin of lipase	supplier	react. time (h)	conversion ^b (%)	e.e. of (<i>S</i>)-2 ^c (%)	<i>E</i>
<i>Pseudomonas</i> sp. ^d	Nagase	22	32	96	77
<i>Cromobacterium viscosum</i> ^e	Toyo Jozo	38	38	93	49
<i>Pseudomonas</i> sp. ^e	Toyobo	38	28	92	34
<i>Pseudomonas</i> sp. ^e	Kurita	20	29	88	22
<i>Pseudomonas fluorescens</i> ^d	Amano	25	57	64	12
<i>Pseudomonas</i> sp. ^e	Toyo Jozo	38	66	47	8

^a Conditions: (\pm)-mandelonitrile (1) (300 mg, 2.25 mmol), isopropenyl acetate (3) (11.27 mmol), lipase (about 30 mg), dry diisopropyl ether (11.3 mL), 40 °C. ^b Determined by GC using ethylbenzene as an internal standard. ^c Determined by the HPLC analysis (CHIRAL CEL OB, hexane(9) : 2-propanol(1), 0.5 mL/min, detected at 254 nm) of the reaction mixture. ^d A commercial lipase preparation was used as received. ^e The lyophilized enzyme powder received was dissolved in 5 mM potassium phosphate buffer and adsorbed on celite diatomite (Hyflo Super-Cel) (see Experimental in Chapter II).

Conversion of the reactions was determined by GC using ethylbenzene as an internal standard. To determine the enantiomeric excesses (e.e.s) of the acetate 2, a small portion of the reaction mixture was analyzed directly on HPLC equipped with a column with chiral stationary phase.

Among the lipase preparations tested, lipase from *Pseudomonas* sp. M-12-33 (Amano Pharm. Co., Ltd.) was highly active. Lipase from *Pseudomonas* sp. (Nagase Biochemicals Co., Ltd.) showed high stereoselectivity based on the enantiomeric ratio (*E*-value):⁵⁴ whereas lipase from *Pseudomonas* sp. (Toyobo Co., Ltd.) was moderate in terms of stereoselectivity. Three lipases from *Pseudomonas* sp. (Amano, Toyobo and Nagase) were selected for the kinetic resolution of racemic mandelonitrile (1) in the preparative scale in diisopropyl ether at 40 °C (Scheme III-1). The results of the kinetic resolution are summarized in Table III-2.

Table III-2 Kinetic Resolution of (\pm)-**1** Catalyzed by Lipase from *Pseudomonas* sp.^a

entry	supplier	react. time (h)	conver- sion ^b (%)	<i>(S)</i> -(-)-acetate 2			<i>(R)</i> -(+)-cyanohydrin 1		
				yield ^c (%)	$[\alpha]_D^d$ (deg)	e.e. ^e (%)	yield ^c (%)	$[\alpha]_D^d$ (deg)	e.e. ^f (%)
1	Toyobo	53	48	46	-5.9 ^g	83	49	+36.4 ^h	78
2	Nagase	79	22	23	-6.8 ⁱ	94	75	+12.8 ^j	27
3 ^k	Amano	65	41	47	-5.8 ^l	80	35	+34.6 ^m	72 ⁿ

^a Conditions: (\pm)-**1** (1.00-5.33 g, 7.51-40.0 mmol), isopropenyl acetate (**3**) (30-80 mmol), lipase (0.76-4.0 g), diisopropyl ether (38-200 mL), 40 °C. ^b Determined by GC using ethylbenzene as an internal standard. ^c Isolated yield based on (\pm)-**1**. ^d Measured in CHCl₃. ^e Determined by ¹H-NMR spectroscopy in the presence of chiral shift reagent, Eu(hfc)₃. ^f Determined by comparing the rotation value with the reported.⁶⁴⁾ ^g c 5.03, at 25 °C. ^h c 5.00, at 22 °C. ⁱ c 5.29, at 21 °C. ^j c 5.01, at 21 °C. ^k The mixture of cyclohexane (**4**) : diisopropyl ether (**1**) was used as solvent. Only in the entry 3, the reaction temperature was 40 °C for 27 h then 23 °C for 38 h. ^l c 5.02, at 24 °C. ^m c 5.06, at 24 °C. ⁿ Determined by the ¹H-NMR spectroscopy of the corresponding MTPA-ester.

At a reasonable conversion of mandelonitrile (**1**) to acetate **2**, the lipase powder was filtered off. The filtrate was concentrated *in vacuo* and chromatographed on silica gel to separate mandelonitrile and its acetate. Both of **1** and **2** were obtained in high chemical yields.

By comparing the optical rotation value of (+)-**1** with the reported value,⁶⁴⁾ the optical purities of (+)-**1** were determined to be 27-78 % e.e. and the absolute configuration as *R* by the (+) sign of the optical rotation. The e.e. of (+)-**1** which determined by the optical rotation (entry 3) was also confirmed by the ¹H-NMR spectroscopy of the corresponding MTPA-ester. The absolute configuration of the acetate (-)-**2** was determined as follows. The acetate (-)-**2** was eluted faster on HPLC using the column with chiral stationary phase than the isomer of the acetate (*R*)-(+)-**2** prepared from the mandelonitrile (*R*)-(+)-**1**. From the differences in retention time on the chromatogram using the chiral column, the acetate (-)-**2** was proved to be *S* configuration.

The optical purities of the acetate (-)-**2** were determined by ¹H-NMR spectroscopy in the presence of a chiral shift reagent, Eu(hfc)₃ (see

experimental). The lipase from *Pseudomonas* sp. (Toyobo, entry 1) attained 48 % conversion in 53 h at 40 °C to afford (*S*)-(-)-**2** with 83 % e.e. in 46 % isolated yield; the remaining (*R*)-(+)-**1** with 78 % e.e. was recovered in 49 % isolated yield. With lipase from *Pseudomonas* sp. (Nagase, entry 2), the reaction proceeded slowly, but the stereoselectivity was high enough to give (*S*)-(-)-**2** in 94 % e.e. The reactivity and stereoselectivity of the lipase from *Pseudomonas* sp. M-12-33 (Amano, entry 3) were almost comparable to those of the lipase from *Pseudomonas* sp. (Toyobo, entry 1), to afford (*S*)-**2** with 80 % e.e. and (*R*)-**1** with 72 % e.e.

In summary, the kinetic resolution of racemic mandelonitrile was accomplished through the lipase-catalyzed transesterification in organic solvent using isopropenyl acetate as an acylating reagent. (*S*)-Mandelonitrile acetate with 80-94 % optical purity was obtained, and the unreacted mandelonitrile with 27-78 % e.e. was also recovered in high chemical yields.

III-3 EXPERIMENTAL

Optical purity of the acetate **2** was analyzed by HPLC using a column with chiral stationary phase (CHIRAL CEL OB, 4.6 ϕ \times 250 mm, Daicel Co, Ltd., hexane (9) : 2-propanol (1), 0.5 mL/min, detected at 254 nm). The retention times of the enantiomers were 32 min for (*S*)-**2** and 40 min for (*R*)-**2**.

Lipase-Catalyzed Stereoselective Acetylation of Racemic 1-Cyano-1-phenylmethanol (**1**); Typical procedure.

1-Cyano-1-phenylmethanol (mandelonitrile) (**1**) (5.33 g, 40 mmol) and isopropenyl acetate (**3**) (8.01 g, 80 mmol) were dissolved in a mixture of cyclohexane (160 mL) and diisopropyl ether (40 mL) in the presence of ethylbenzene (1.06 g, 10 mmol) as an internal standard for GC analysis. Lipase from *Pseudomonas* sp. M-12-33 (Amano Pharm. Co., Ltd.) (4.0 g) was suspended into the solution. The suspension was stirred for 27 h at 40 °C and then for 38 h at 23 °C. The reaction mixture was filtered and the lipase powder was washed twice with diisopropyl ether. The combined

filtrates were concentrated *in vacuo* and chromatographed on silica gel [hexane:AcOEt = 12:1 to 10:1] to afford (*S*)-(-)-**2**: (3.27 g, 47 % yield) and (*R*)-(+)-**1**: (1.85 g, 35 % yield).

(*R*)-(+)-Mandelonitrile (1**).**

$[\alpha]_D^{24} = +34.6^\circ$ (*c* 5.06, CHCl₃); (Lit.⁶⁴) $[\alpha]_D^{25} = +43.5^\circ$ (*c* 5, CHCl₃) for

the *R* isomer with 92.5 % e.e.); ¹H-NMR δ 3.42 (d, 1H, *J* = 6.8 Hz, OH), 5.51 (d, 1H, *J* = 6.8 Hz, CH), and 7.38-7.55 (m, 5H_{arom}). The e.e. was determined to be 72 % by the ¹H-NMR spectroscopy of the corresponding MTPA-ester. A pair of the multiplet signals of methoxy proton were base-line separated [δ (OMe) 3.46 for (*R*)-**1** and 3.58 for (*S*)-**1**].

(*S*)-(-)-Mandelonitrile acetate (2**).**

$[\alpha]_D^{24} = -5.8^\circ$ (*c* 5.02, CHCl₃); ¹H-NMR δ 2.17 (s, 3H, OAc), 6.41 (s, 1H, CH), and 7.42-7.56 (m, 5H_{arom}). The e.e. was determined to be 80 % by the ¹H-NMR spectroscopy in the presence of chiral shift reagent, Eu(hfc)₃. A pair of the singlet signals of acetyl group were base-line separated [δ (OAc) 2.88 for (*S*)-**2** and 3.00 for (*R*)-**2**].

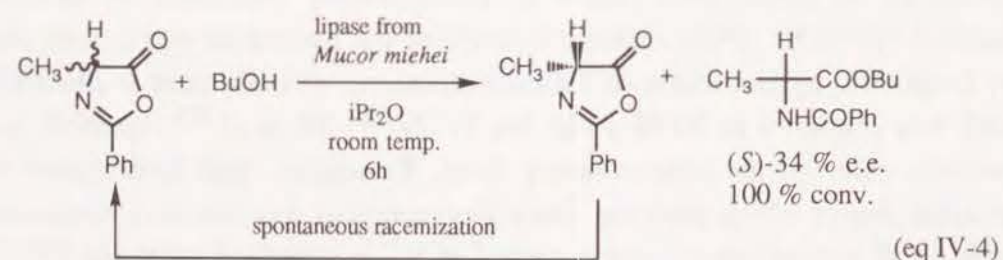
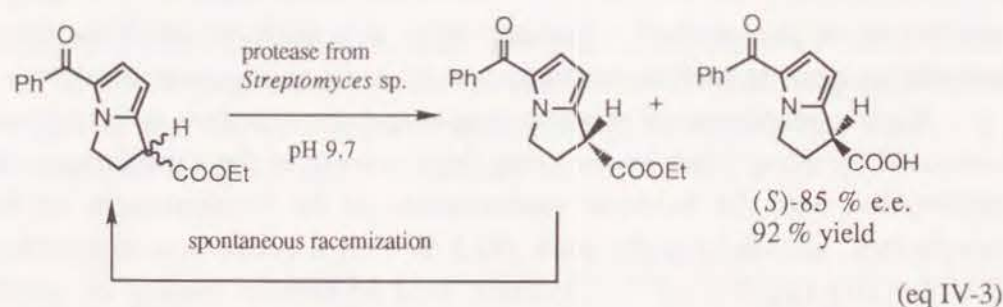
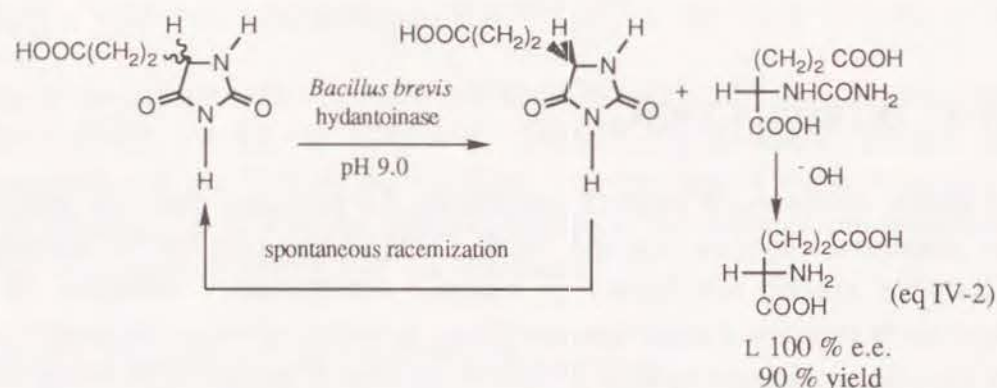
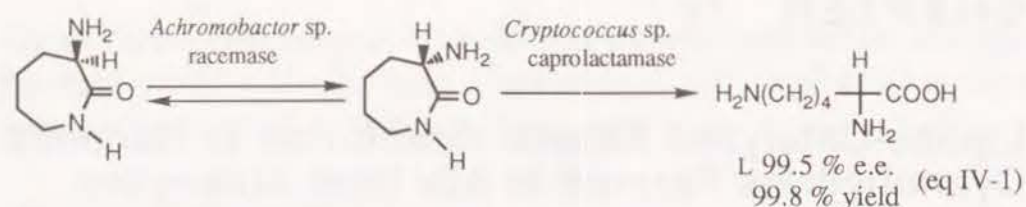
CHAPTER IV

Lipase-Catalyzed Kinetic Resolution of Racemic Cyanohydrins Formed *in situ* from Aldehydes

IV-1 INTRODUCTION

In kinetic resolution of racemic compounds, the maximum chemical yield of an enantiomer does not exceed 50 %. This is also true for the enzyme-catalyzed kinetic resolutions of racemic compounds. However, if a reaction is conducted under the conditions in which substrate are racemized *in situ*, the yield could exceed 50 % without loss of optical yield and hence, the racemic substrates could be converted completely into a single enantiomer of the product. Racemization is usually attained by using isomerizing enzyme such as racemase and by using acid or base-catalysts.

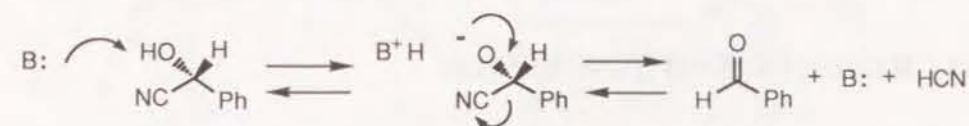
Kinetic resolution of racemic α -amino- ϵ -caprolactam to lysine was conducted by using yeast hydrolysing the L-isomer of the caprolactam. In combination with the bacterial racemization of the D-enantiomer of the caprolactam, almost optically pure (99.5 % e.e.) L-lysine was obtained in 99.8 % yield (eq IV-1).⁶⁵ Glutamic acid hydantoin having an acidic proton on its asymmetric center is spontaneously racemized in alkaline medium (pH >9). When racemic mixture of the hydantoin was hydrolyzed by D-specific hydantoinase in alkaline solution, optically pure D-glutamic acid was prepared in 90 % yield (eq IV-2).⁶⁶ Sih et al.⁶⁷ reported that racemic ester of an inflammatory drug, Ketorolac, was hydrolyzed in alkaline buffer using protease from *Streptomyces griseus* and Ketorolac with 85 % optical purity was obtained in 92 % chemical yield (eq IV-3). Only one example, in which racemic isomers were subjected to non-enzymatic racemization, has been reported for lipase-catalyzed resolution in organic solvent by Bevinakatti et al.⁶⁸ Racemic oxazolones derived from *N*-benzoylalanine were resolved by lipase-catalyzed transesterification with 1-butanol. (*S*)-*N*-Benzoylalanine butyl ester obtained was 34 % e.e. at 100 % conversion (eq IV-4).



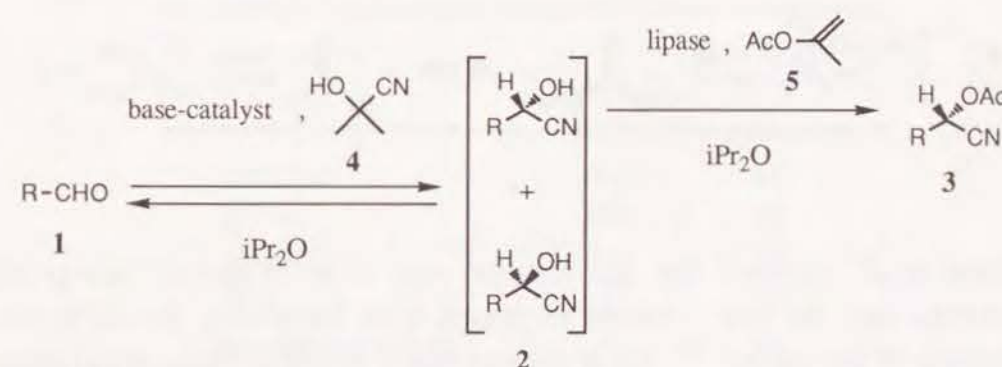
In Chapter III, kinetic resolution of racemic 1-cyano-1-phenylmethanol (mandelonitrile) was accomplished by the lipase-catalyzed stereoselective acetylation using isopropenyl acetate in diisopropyl ether.

It was observed that an optically active sample of mandelonitrile dissolved in diisopropyl ether lost its optical activity within a day under the presence of catalytic amount of triethylamine. This racemization was

supposed to be attributed to the triethylamine-catalyzed elimination of hydrogen cyanide from mandelonitrile as previously proposed by Inoue et al.⁶⁹⁾ in a cyclic dipeptide-catalyzed asymmetric addition of hydrogen cyanide to aldehydes.



If pre-equilibrium between cyanohydrin and aldehyde was attained rapidly by base-catalysts before the lipase-catalyzed stereoselective acylation, the kinetic resolution of racemic cyanohydrins could proceed over 50 % chemical yield with maintaining high optical purity (Scheme IV-1).



Scheme IV-1 Design of Lipase-Catalyzed Kinetic Resolution of Racemic Cyanohydrins formed *in situ* from Aldehydes

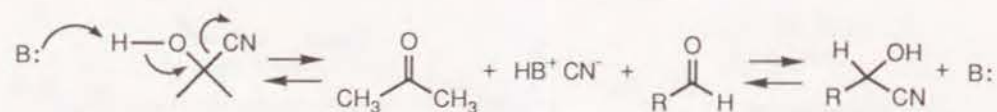
2-Cyano-2-propanol (acetone cyanohydrin) (4) was selected as a hydrogen cyanide source, because this is less toxic, easy to handle than hydrogen cyanide and soluble in diisopropyl ether. Acetone cyanohydrin releases hydrogen cyanide in the presence of base-catalysts and reacts with

aldehydes giving cyanohydrins.^{69, 70, 71, 72, 73}) There are several other advantages of using acetone cyanohydrin; after the release of cyanide, acetone cyanohydrin gives acetone which has no adverse effect on lipase. Acetone cyanohydrin is not acetylated by lipase-catalyzed transesterification because it is tertiary alcohol and not accepted as a substrate by lipase.⁹⁾

IV-2 RESULTS AND DISCUSSION

Screening of Base-Catalysts for Cyanohydrin Formation from Aldehyde and Acetone Cyanohydrin

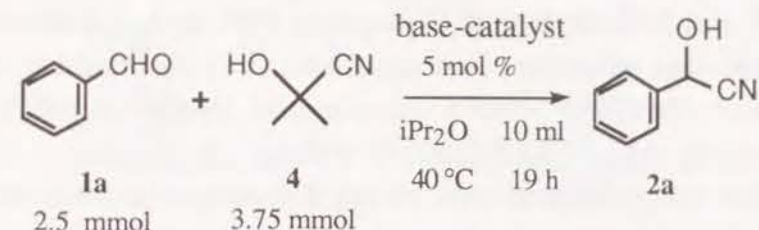
Base-catalyzed cyanohydrin formation is considered to proceed as follows: acetone cyanohydrin decomposes to acetone and cyanide ion complex with base, then the cyanide ion adds to aldehyde.



Ogata et al. reported that the reaction was slow in aprotic non-polar solvents, and the rate constant increased with increasing the dielectric constant of the solvent.⁷⁴⁾ In the present study, however, diisopropyl ether was used as a solvent. Therefore an effective base-catalyst was required to accelerate the reaction in this non-polar solvent. Cinchona alkaloids such as quinidine (**6a**) and its diastereoisomer, quinine (**6b**) were used as a catalyst for the addition of HCN to benzaldehyde.⁷⁵⁾ Cinchona alkaloids are a bi-functional catalyst because their tertiary aminogroup works as a base and the hydroxy group serves as an acid.⁷⁶⁾

Six bases including cinchona alkaloids were tested for their catalytic activity for the mandelonitrile formation from benzaldehyde and acetone cyanohydrin. Benzaldehyde (**1a**) allowed to react with 1.5 equivalent

moles of acetone cyanohydrin (**4**) in diisopropyl ether in the presence of 5 mol % of base-catalysts (Scheme IV-2).



Scheme IV-2 Screening of Base-Catalysts for Mandelonitrile Formation from Benzaldehyde and Acetone Cyanohydrin

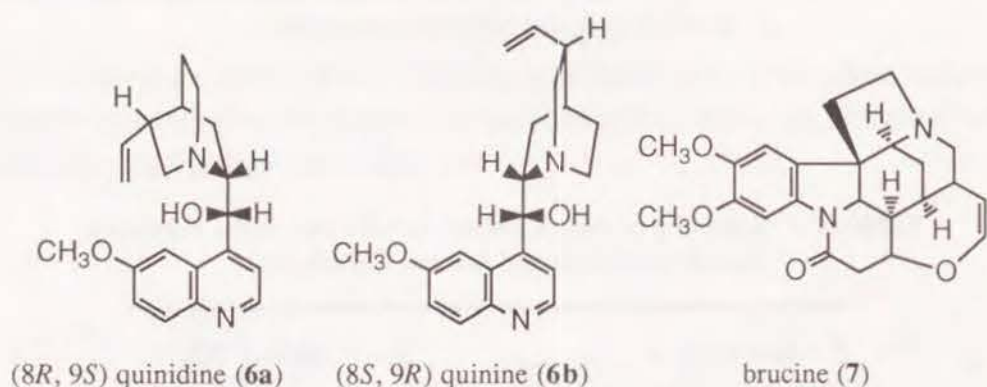
Table IV-1 Screening of Base-Catalysts for Mandelonitrile Formation from Benzaldehyde and Acetone Cyanohydrin^a

base-catalyst		yield of 2a ^b (%)
quinidine	(6a)	85 ^c
quinine	(6b)	83 ^c
brucine	(7)	20 ^c
NEt ₃	(8)	58
Me ₂ NCH ₂ CH ₂ OH	(9)	34
Et ₂ NCH ₂ CH(CH ₃)OH	(10)	25
lipase ^d		0
none		0

^a Conditions: Benzaldehyde (**1a**) (2.5 mmol), 2-cyano-2-propanol (**4**) (3.75 mmol), base-catalyst (0.125 mmol), iPr₂O (10 mL), 40 °C.

^b Determined by ¹H-NMR. ^c The compound **2a** formed was converted into the acetate **3a** and the e.e. of **3a** was determined as 0 % by ¹H-NMR using a chiral shift reagent, Eu(hfc)₃. ^d The lipase preparation from *Pseudomonas* sp. M-12-33 (Amano) (25 mg) was added.

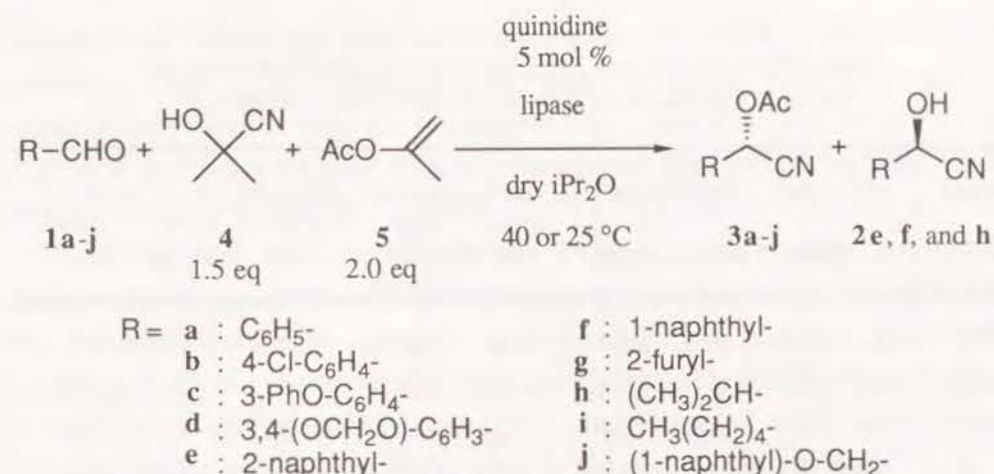
Among the bases tested (Table V-1), quinidine (**6a**) and quinine (**6b**) afforded mandelonitrile (**2a**) at 85 and 83 % conversion yield in 19 h. Under the same reaction conditions, brucine (**7**) and triethylamine (**8**) gave only 58 and 20 % of mandelonitrile, respectively. 2-Dimethylamino-ethanol (**9**) and 3-diethylamino-2-propanol (**10**) having β -aminoalcohol portion were less effective than quinidine (**6a**) and quinine (**6b**). No reaction was observed with a commercial lipase preparation from *Pseudomonas* sp. M-12-33 (Amano) or without a base-catalyst, confirming that lipase has no catalytic activity for the formation of mandelonitrile.



Since quinidine (**6a**) and quinine (**6b**) are chiral base-catalysts, it was anticipated that the addition of cyanide ion was stereoselective and hence that mandelonitrile produced was optically active. Mandelonitrile formed through the catalysis by **6a** and **6b**, however, was racemic.

Preliminary Experiment for Kinetic Resolution of Racemic Cyanohydrins Formed *in situ* from Aldehydes

In order to obtain optically active cyanohydrin acetates with high enantiomeric excess, it was necessary to find out the lipase showing high stereoselectivity. Lipases from *Pseudomonas* sp.s (Amano and Toyobo Co.) were tested for aldehydes **1a-j** (Scheme IV-3).



Scheme IV-3 Lipase-Catalyzed Kinetic Resolution of Racemic Cyanohydrins formed *in situ* from Aldehydes

The proceeding of the reactions was checked by ¹H-NMR with monitoring the signals of aldehyde proton of the substrate aldehydes **1**, methyne proton of cyanohydrins **2**, and methyne proton of the acetates **3**. Fig IV-1 shows a typical ¹H-NMR spectrum of the reaction mixture.

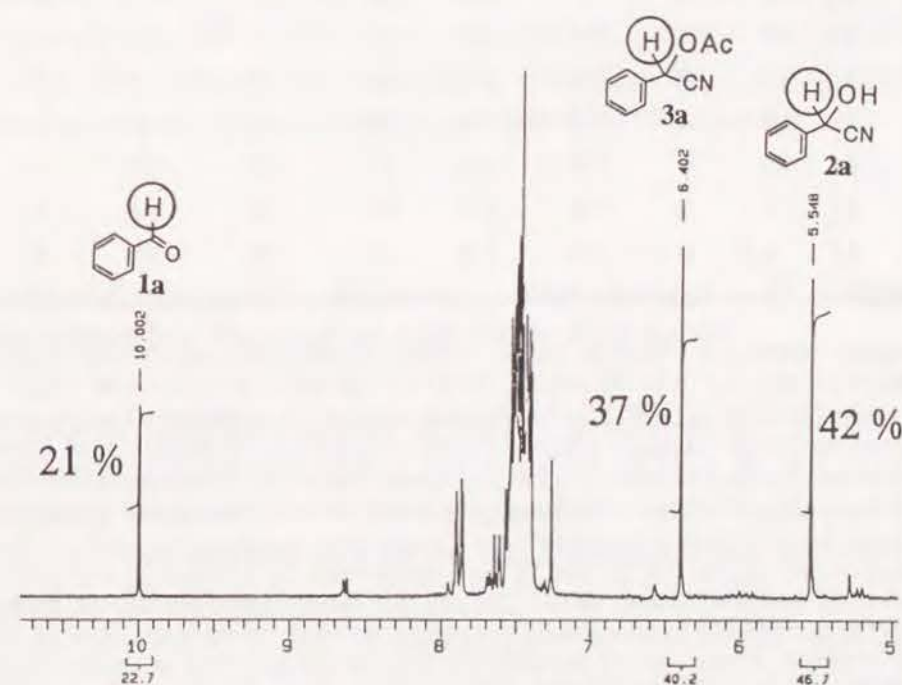


Fig. IV-1 ¹H-NMR spectrum of the reaction mixture of **1a**.

Table IV-2 Preliminary Experiment for Kinetic Resolution of Racemic Cyanohydrins **2** formed *in situ* from Aldehydes **1a**

entry	aldehyde		lipase prep. ^b		react. time (d)	conver- sion ^c (%)	isolated yield ^d (%)	e.e. of 3 ^e (%)	absolute config. ^f
	(mmol)	abb.	(mg)						
1	1a	40	A	2000	1.6	37	34	83	<i>S</i>
2	1a	5	B	250	4.0	28	26	54	<i>S</i>
3	1b	10	A	500	5.9	45	39	81	<i>S</i>
4	1b	5	B	250	4.0	25	17	83	<i>S</i>
5	1c	10	A	500	2.5	38	20	83	<i>S</i>
6	1c	0.4	B	10	5.0	27	23	85	<i>S</i>
7	1d	5	A	250	4.1	25	22	95	<i>S</i>
8	1d	0.6	B	30	1.7	34	27	81	<i>S</i>
9	1e	10	A	1330	2.8	45	43	85	<i>S</i>
10	1e	1	B	50	1.8	34	30	91	<i>S</i>
11	1f	10	A	666	6.7	40	36	69	<i>S</i>
12	1f	1	B	50	1.8	25	20	93	<i>S</i>
13	1g	12	A	600	2.0	45	42	47	<i>R</i>
14	1g	12	B	360	2.7	33	26	52	<i>R</i>
15 ^g	1h	15	A	200	3.0	21	15	69	<i>S</i>
16 ^g	1h	15	B	150	3.0	25	15	71	<i>S</i>
17 ^g	1i	15	A	200	2.0	27	11	34	<i>S</i>
18 ^g	1i	15	B	150	3.4	33	27	75	<i>S</i>
19	1j	1.5	A	100	2.8	44	28	74	<i>R</i>
20	1j	1.5	B	100	2.8	62	38	48	<i>R</i>

^a Conditions: Aldehyde **1** (0.4-40 mmol), acetone cyanohydrin (**4**) (0.6-60 mmol), isopropenyl acetate (**5**) (0.8-80 mmol), iPr₂O (5-160 mL), quinidine (**6a**) (0.02-2 mmol), lipase (10-2000 mg), 40 °C. ^b Lipases used were: A, commercial preparation from *Pseudomonas* sp. M-12-33 (Amano Pharm. Co., LTD.). B, immobilized lipase from *Pseudomonas* sp. (Toyobo Co., Ltd.) on Hyflo Super-Cel. ^c Determined by ¹H-NMR of the reaction mixture. ^d Isolated yield based on **1**. ^e Determined by the ¹H-NMR spectroscopy in the presence of a chiral shift reagent, Eu(hfc)₃. ^f The configurations of **2a**, **2c**, **2d**, **2i**, and **2j** were determined by comparing the optical rotation values with the reported ones. The absolute configurations of **2b**, **2e**, **2f**, **2g**, and **2h** were determined by comparing the optical rotation values with those of the authentic samples. Preparation of the authentic samples are described in Experimental section of this chapter. ^g Reaction temperature was 25 °C.

The reactions were terminated when the conversion yield of the acetates **3** reached 21-65 %. Then, the reaction mixture was filtered and concentrated filtrate was chromatographed on silica gel to afford the acetates **3** and cyanohydrins **2**. Only the conversion yields, isolated yields, and e.e.s of **3** are shown in Table IV-2.

All the reactions were clean: side reactions such as benzoin condensation or acetylation of quinidine were not observed at all. ¹H-NMR also demonstrated that acetone cyanohydrin (**4**) was not acetylated. Quinidine did not catalyze the acetylation of mandelonitrile (**2a**), because the formation of the acetate (**3a**) was not detected after a prolonged incubation of **2a** with isopropenyl acetate (**5**) and quinidine (**6a**).

The e.e. of the acetates **3a-j** were determined by ¹H-NMR spectroscopy in the presence of a chiral shift reagent, Eu(hfc)₃ (see Experimental). The absolute configurations of **3a**, **c**, **d**, **i**, and **j** were determined by comparing the observed optical rotation with reported ones.^{56, 77, 78, 79} Since the optical rotation values have not been reported for **3b**, **e**, **f**, **g**, and **h**, the corresponding cyanohydrins **2**, the absolute configuration of which is known, were isolated from the reactions of Scheme IV-3 or prepared from another route (see Experimental). Then the optically active cyanohydrins having known configuration were acetylated to afford the authentic sample of the acetates for comparison. By considering both of the conversion and e.e., lipase from *Pseudomonas* sp. M-12-33 (Amano) was selected for aldehydes **1b**, **c**, **d** and lipase from *Pseudomonas* sp. (Toyobo) was also selected for aldehyde **1e**.

Lipase-Catalyzed Kinetic Resolution of Racemic Cyanohydrins Formed *in situ* from Aldehydes

In order to ascertain that this kinetic resolution proceeds with *in situ* racemization of cyanohydrins **2**, the reactions of **1b**, **c**, **d**, and **e** were kept going until the conversion of the reactions exceeded 50 % (Table IV-3). The best result was obtained for the reaction of aldehyde **1d** catalyzed by lipase from *Pseudomonas* sp. M-12-33 (Amano) (entry 3): after 13.7 days, acetate **3d** with 82 % e.e. was obtained in 69 % isolated yield based on aldehyde **1d**. Since 20 % of aldehyde **1d** was remained in the reaction mixture, the total conversion yield of the cyanohydrin **2d** from aldehyde

1d should be 80 %. After the correction, the conversion from cyanohydrin **2d** to acetate **3d** was virtually reached 91 %, while high optical yield was retained.

Table IV-3 Lipase-Catalyzed Kinetic Resolution of Racemic Cyanohydrins **2** formed *in situ* from Aldehydes **1a**

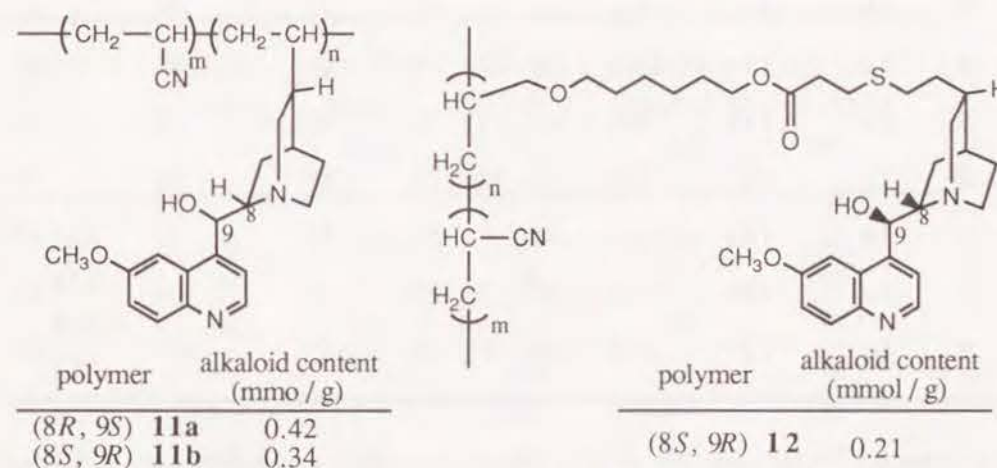
entry	aldehyde	lipase prep. ^b	react. time (d)	conversion ^c 1 : 2 : 3 (%)	conversion from 2 to 3 ^d (%)	isolated yield of 3 ^e (%)	e.e. of (S)-3 ^f (%)
		abb. (mg)					
1	1b	A 100	16.6	8 20 72	78	40	61
2	1c	A 150	13.7	19 10 71	88	68	62
3 ^g	1d	A 200	16.7	20 7 73	91	69	82
4	1e	B 200	4.4	6 3 91	97	89	40

^a Aldehyde **1b-d** (1-2 mmol), acetone cyanohydrin (**4**) (1.5-3.0 mmol), isopropenyl acetate (**5**) (2.0-4.0 mmol), quinidine (**6a**) (0.05-0.10 mmol), lipase (100-200 mg), iPr₂O (8-16 mL), 40 °C. ^b Lipases used were: A, from *Pseudomonas* sp. M-12-33 (Amano); B, immobilized lipase from *Pseudomonas* sp. (Toyobo) on Hyflo Super-Cel. ^c Determined by ¹H-NMR. ^d Calculated conversion yield of the acetate **3** based on the cyanohydrin **2** formed *in situ*. ^e Isolated yield based on **1**. ^f Determined by ¹H-NMR in the presence of a chiral shift reagent, Eu(hfc)₃. ^g 2 mmol of **1c** was used.

Also, the reaction of entry 1, 2, and 4, in which the conversion from cyanohydrins **2** to acetates **3** reached 78-97 %, gave optically active acetates (S)-**3** with 40-62 % e.e. These results indicate that the enantiomer of cyanohydrin which was not available to lipase was racemized during the lipase-catalyzed kinetic resolution.

Application of Polymer-Supported Quinidine and Quinine as Catalyst for Cyanohydrin Formation

One of the methods to improve the convenience of handling catalysts is to immobilize catalyst onto insoluble polymer matrix. Polymer-supported cinchona alkaloids have been prepared.^{80, 81, 82, 83, 84} According to the reported procedure,^{80, 84} the polymer-supported quinidine **11a**, quinine **11b**, and quinine having a spacer group of 15 atoms-length **12** were prepared and used as a catalyst for *in situ* formation of cyanohydrin in the kinetic resolution catalyzed by lipase from *Pseudomonas* sp. M-12-33 (Amano) using aldehydes **1b-d** (Scheme IV-3). The content of alkaloid moiety in the polymers was determined by elemental analysis. The polymers were added so that the alkaloid content could be 5 mol % to aldehydes **1**.



None of the polymer-catalyst was soluble in diisopropyl ether, and the reaction proceeded in heterogeneous. The results are summarized in Table IV-4. In entry 1 using polymer **11a**, (S)-**3b** with 81 % e.e. was obtained when the conversion from **2b** to **3b** was 85 %. In entry 3 using polymer **12**, (S)-**3c** with 74 % e.e. was afforded at 86 % conversion. The reaction of **1d** was slower than those of **1b** and **1c** as observed in the reactions catalyzed by quinidine (Table IV-2, entry 7 and 8). However, the stereoselectivity was high and (S)-**3d** with 86-92 % e.e. was obtained at 79-85 % conversion. Optical purities and yields of the reactions with

polymer-catalysts **11-12** were almost equal to those catalyzed by quinidine (**6a**).

Table IV-4 Application of Polymer-Supported Cinchona Alkaloid **11a, b**, and **12** for Lipase-Catalyzed Kinetic Resolution of Racemic Cyanohydrins **2** Formed *in situ* from Aldehydes **1a**

entry	aldehyde	polymer catalyst ^b	react. time (d)	conversion ^c 1 : 2 : 3 (%)			conversion from 2 to 3 ^d (%)	isolated yield of 3 ^e (%)	e.e. of (<i>S</i>)- 3 ^f (%)
1	1b	11a	13.7	9	14	77	85	54	81
2	1b	11b	8.6	16	22	62	74	45	87
3	1b	12	19.4	17	23	60	72	40	74
4	1c	11a	11.5	14	12	74	86	61	69
5	1c	11b	8.6	13	15	72	83	63	64
6	1c	12	13.0	13	12	75	86	50	74
7	1d	11a	13.7	38	9	53	85	51	86
8	1d	11b	11.5	37	13	50	79	34	92
9	1d	12	15.8	39	9	52	85	41	88

^a Conditions: aldehydes **1b-d** (1.0 mmol), acetone cyanohydrin (**4**) (1.5 mmol), isopropenyl acetate (**5**) (2.0 mmol), polymer **11a, b**, and **12** (0.05 mmol equiv.), iPr₂O (8 mL), 40 °C. ^b Alkaloid content and amount of polymer were: **11a**, 0.44 mmol / g, 120 mg; **11b**, 0.34 mmol / g, 147 mg; **12**, 0.21 mmol / g, 240 mg. ^c Determined by ¹H-NMR. ^d Calculated conversion yield of the acetate **3** based on the cyanohydrin **2** formed *in situ*. ^e Isolated yield based on **1**. ^f Determined by ¹H-NMR in the presence of a chiral shift reagent, Eu(hfc)₃.

As discussed in the section of screening of base-catalysts, quinidine (**6a**) and quinine (**6b**) were interchangeable as a catalyst for this reaction. In fact, the results using polymer **11a** (quinidine) and **11b** (quinine) were

comparable. Although the polymer **12** has a spacer group of 15 atom-length between quinine portion and the polymer chain, no remarkable difference was observed on the conversion yield and e.e. of (*S*)-acetate.

A major advantage of the immobilization of catalyst is that the catalyst can be recovered and recycled. Thus, the polymer **11b** and **12** were recovered together with the lipase powder, and the mixture was dried in a desiccator (over P₂O₅, more than 3 days) and reused for the reaction with aldehyde **1c** (Table IV-5). Polymer **11b** and **12** had almost the same catalytic activity after three or four times of use. This anticipated providing the possibility of building a reactor system for continuous production of optically active cyanohydrin acetates **3**.

Table IV-5 Recycle Use of Polymer **11b** and **12** for Kinetic Resolution of Racemic Cyanohydrin **2c** formed *in situ* from Aldehyde **1c**^a

polymer	recycle numbers	reaction time (days)	conversion ^b (%)	isolated yield of 3c ^c (%)	e.e. of of (<i>S</i>)- 3c ^d (%)
11b	1st	2.0	38	35	85
11b	2nd	2.0	29	16	94
11b	3rd	2.0	28	19	90
12	1st	2.0	42	23	88
12	2nd	2.1	44	43	85
12	3rd	2.0	62	52	87
12	4th	2.0	42	38	85

^a Polymer **11b** (294 mg, 0.1 mmol equiv.) or **12** (480 mg, 0.1 mmol equiv.) and a lipase from *Pseudomonas* sp. M-12-33 (150 mg) were used at the first run. Every 2.0 days, the polymer and lipase were recovered together by filtration and dried in a desiccator over P₂O₅ for more than 2 days, and then reused. Conditions: 3-Phenoxybenzaldehyde (**1c**) (397 mg, 2 mmol), acetone cyanohydrin (**4**) (222 mg, 2.6 mmol), isopropenyl acetate (**5**) (330 mg, 3.3 mmol), dry iPr₂O (8 mL) 40 °C 2.0 days. ^b Determined by ¹H-NMR. ^c Isolated yield based on **1c**. ^d Determined by the ¹H-NMR spectroscopy in the presence of a chiral shift reagent, Eu(hfc)₃.

In summary, a new system for kinetic resolution of racemic cyanohydrins with *in situ* racemization has been established: racemic cyanohydrins were generated by the quinidine-catalyzed reaction from aldehydes and acetone cyanohydrin, then stereoselectively acetylated with isopropenyl acetate in the presence of lipase. As one of the enantiomers of cyanohydrin was selectively transformed into acetate, the enantiomer which was available to lipase was supplied from the other one through the addition-elimination equilibrium between aldehyde and acetone cyanohydrin. Accordingly the chemical yield of the optically active cyanohydrin acetates exceeded 50 %. By supporting quinidine and quinine on polymer, recycle use of the base-catalysts and lipases was attained. This new system will serve as a more convenient and versatile route for preparation of optically active cyanohydrin acetates.

IV-3 EXPERIMENTAL

The starting aldehydes **1a-i** were commercially available and purified before use by distillation or recrystallization under an argon atmosphere. The aldehyde **1j** was prepared according to the reported procedure,⁵⁶⁾ and its purity was ascertained by ¹H-NMR. The commercial lipase preparation from *Pseudomonas* sp. M-12-33 (Amano Pharm. Co., Ltd.) was used as such. The lyophilized powder of lipase from *Pseudomonas* sp. (Toyobo Co., Ltd.) was immobilized by adsorption onto diatomaceous earth, Hyflo Super-Cel, according to the procedure described in Chapter II, except that 20 mM Tris-HCl buffer (pH 8.0) was used as solubilizing buffer.

Lipase-Catalyzed Kinetic Resolution of Racemic Cyanohydrins Formed *in situ* from Aldehydes

(S)-(-)-1-Cyano-1-phenylmethyl acetate (**3a**); Typical Procedure.

Benzaldehyde (**1a**) (4.24 g, 40 mmol), isopropenyl acetate (8.01 g, 80 mmol), 2-cyano-2-propanol (5.11 g, 60 mmol) and quinidine (649 mg, 2 mmol) were dissolved in dry diisopropyl ether (160 mL). Lipase from *Pseudomonas* sp. M-12-33 (Amano) (2.00 g) was added to the solution, and the suspension was stirred for 39 h at 40 °C under an argon atmosphere.

The lipase powder was filtered off and the filtrate was concentrated *in vacuo*. A portion of the residual oil was analyzed by ¹H-NMR. Three singlets [CHO proton for the aldehyde **1a** (δ = 10.00), CH proton for the cyanohydrin **2a** (δ = 5.55), and CH proton for the acetate **3a** (δ = 6.40)] were clearly separated; the composition of the oil was calculated to be **1a** (21 %), **3a** (37 %), and **2a** (42 %). The acetate **3a** was isolated from the mixture by flash column chromatography [hexane (5) : AcOEt (1)] as a colorless oil: (2.36 g, 34 %); $[\alpha]_D^{25}$ = +19.3° (*c* 2.230, benzene), [lit.⁷⁸⁾ $[\alpha]_D$ = -15° (*c* 1.9, benzene) for *R* isomer with 60 % e.e.]. Optical purity of (+)-**3a** was determined as 83 % by ¹H-NMR in the presence of a chiral shift reagent Tris[3-(heptafluoropropylhydroxymethylene)-(+)-camphorato], europium (III) derivative, Eu(hfc)₃ [ca. 10 mg for 5 mg of **3a** in 800 μ l of CDCl₃; δ (OAc) 3.01 (*R*) and 3.14 (*S*)]. IR (neat) 2250 (C \equiv N) and 1755 (C=O) cm⁻¹; ¹H-NMR δ 2.17 (s, 3H, OAc), 6.41 (s, 1H, CH), and 7.42-7.56 (m, 5H_{arom}); ¹³C-NMR δ 20.47 (CH₃CO), 62.85 (CH), 116.11 (C \equiv N), 127.88, 129.25, 130.41, 131.74, and 168.93 (C=O); MS (70 eV) *m/z* (relative intensity %) 175 (M⁺, 6), 133 (35), 116 (28), 115 (41), 105 (16), 89 (10), and 43 (100). Anal. Calcd. for C₁₀H₉NO₂: C, 68.56; H, 5.18; N, 8.00 %. Found: C, 68.34; H, 5.23; N, 7.90 %.

Compounds **3b-j** were prepared by the same procedure from the corresponding aldehydes **1b-j**. Only the starting aldehyde, purification method, yield, physical and spectroscopic data are given for each cyanohydrin acetate **3b-j**.

(S)-(+)-1-Cyano-1-(4-chlorophenyl)methyl acetate (**3b**).

Prepared from 4-chlorobenzaldehyde (**1b**). Flash column chromatography on silica gel eluting with [hexane (8) : AcOEt (1)] gave a colorless oil: (818 mg, 39 %); $[\alpha]_D^{25}$ = +31.2° (*c* 2.08, benzene); 81 % e.e. [¹H-NMR using Eu(hfc)₃; δ (OAc) 2.34 (*R*) and 2.36 (*S*)]; IR (neat) 2250 (C \equiv N) and 1755 (C=O) cm⁻¹; ¹H-NMR δ 2.17 (s, 3H, OAc), 6.38 (s, 1H, CH), and 7.39-7.52 (m, 4H_{arom}); ¹³C-NMR δ 20.41 (CH₃CO), 62.16, (CH), 115.81 (C \equiv N), 129.28, 129.50, 130.29, 136.58, and 168.82 (C=O); MS (70 eV) *m/z* (relative intensity %) 209 (M⁺, 6), 211 ([M+2]⁺, 2), 167 (21), 149 (32), 114 (14), and 43 (100); Anal. Calcd. for C₁₀H₈ClNO₂: C, 57.30; H, 3.85; N, 6.68 %. Found: C, 57.46; H, 3.88; N, 6.87 %. The absolute configuration of (+)-**3b** was determined by comparing its optical

rotation with that of the authentic sample (*R*)-(-)-**3b** derived from the optically active cyanohydrin (*R*)-(+)-**2b** with a known configuration (*vide infra*).

(*S*)-(+)-1-Cyano-1-(3-phenoxyphenyl)methyl acetate (3c).

Prepared from 3-phenoxybenzaldehyde (**1c**). Flash column chromatography on silica gel eluting with [hexane (6) : AcOEt (1)] followed by rechromatography eluting with [hexane (40) : AcOEt (1)] gave a colorless oil: (540 mg, 20 %); $[\alpha]_D^{27} = +21.25^\circ$ (*c* 10.27, benzene) [lit.⁷⁷ $[\alpha]_D^{20} =$

$+17.1^\circ$ (*c* 10, benzene) for *S* isomer]; 83 % e.e. [¹H-NMR using Eu(hfc)₃; δ (OAc) 2.34 (*R*) and 2.36 (*S*)]; IR (neat) 2250 (C≡N) and 1760 (C=O) cm⁻¹; ¹H-NMR δ 2.17 (s, 3H, OAc), 6.36 (s, 1H, CH), and 6.99-7.44 (m, 9H_{arom}); ¹³C-NMR δ 20.45 (CH₃CO), 62.43 (CH), 115.90 (C≡N), 117.69, 119.39, 120.09, 122.10, 124.11, 130.00, 130.64, 133.45, 156.18, 158.20, and 168.83 (C=O); MS (70 eV) *m/z* (relative intensity %) 267 (M⁺, 52), 225 (100), 206 (7), 197 (11), 181 (19), 147 (12), 114 (36), 77 (29), 51 (25), and 43 (49); Anal. Calcd. for C₁₆H₁₃NO₃: C, 71.90; H, 4.90; N, 5.24 %. Found: C, 71.81; H, 4.92; N, 5.48 %.

(*S*)-(+)-1-Cyano-1-(3,4-methylenedioxyphenyl)methyl acetate (3d).

Prepared from 3,4-(methylenedioxy)benzaldehyde (**1d**). Preparative thin layer chromatography on silica gel developed with [hexane (5) : AcOEt (1)] for 3 times yielded **3d** as a colorless oil: (35.7 mg, 27 %); $[\alpha]_D^{25} = +36.9^\circ$ (*c* 1.77, benzene) [lit.⁷⁸ $[\alpha]_D = -44^\circ$ (*c* 1.7, benzene) for *R* isomer with 99.5 % e.e.]; 85 % e.e. [¹H-NMR using Eu(hfc)₃; δ (OAc) 2.50 (*R*) and 2.56 (*S*)]; IR (neat) 2250 (C≡N) and 1755 (C=O) cm⁻¹; ¹H-NMR δ 2.15 (s, 3H, OAc), 6.03 (s, 2H, OCH₂O), 6.31 (s, 1H, CH), 6.84 (m, 1H, 6'-H), 6.99 (m, 1H, 2'-H), and 7.01 (m, 1H, 5'-H); ¹³C-NMR δ 20.50 (CH₃CO), 62.67 (CH), 101.79 (OCH₂O), 108.22, 108.64, 116.15 (C≡N), 122.44, 125.36, 148.41, 149.40, and 168.92 (C=O); MS (70 eV) *m/z* (relative intensity %) 219 (M⁺, 88), 177 (100), 160 (99), 159 (100), 149 (29), 130 (21), 129 (22), 102 (34), 75 (32), 63 (28), 51 (28), and 43 (86); Anal. Calcd. for C₁₁H₉NO₄: C, 60.28; H, 4.14; N, 6.39 %. Found: C, 59.98; H, 4.25; N, 6.53 %.

(*S*)-(+)-1-Cyano-1-(2-naphthyl)methyl acetate (3e).

Prepared from 2-naphthaldehyde (**1e**). Flash column chromatography on silica gel eluting with [hexane (8) : AcOEt (1)] and followed by rechromatography eluting with hexane (12) : AcOEt (1) gave **3e** as a white crystalline solid: (968 mg, 43 %); mp. 35 °C; $[\alpha]_D^{25} = +21.7^\circ$ (*c* 1.01, CHCl₃); 85 % e.e. [¹H-NMR using Eu(hfc)₃; δ (OAc) 2.66 (*R*) and 2.71 (*S*)]; IR (KBr) 2245 (C≡N) and 1755 (C=O) cm⁻¹; ¹H-NMR δ 2.18 (s, 3H, OAc), 6.58 (s, 1H, CH), 7.50-7.61 (m, 3H_{arom}), 7.82-7.95 (m, 3H_{arom}), and 8.02 (m, 1H_{arom}); ¹³C-NMR δ 20.52 (CH₃CO), 63.07 (CH), 116.17 (C≡N), 124.28, 127.09, 127.59, 127.83, 128.02, 128.38, 128.92, 129.45, 132.85, 133.88, and 168.99 (C=O); MS (70 eV) *m/z* (relative intensity %) 225 (M⁺, 31), 183 (80), 166 (63), 165 (100), 155 (19), 139 (21), 127 (26), and 43 (72); Anal. Calcd. for C₁₄H₁₁NO₂: C, 74.65; H, 4.92; N, 6.22 %. Found: C, 74.65; H, 4.93; N, 6.25 %. The absolute configuration of (+)-**3e** was determined by comparing its optical rotation with that of the authentic sample (*R*)-(-)-**3e** derived from the optically active cyanohydrin (*R*)-(+)-**2e** having a known configuration (*vide infra*).

(*S*)-(-)-1-Cyano-1-(1-naphthyl)methyl acetate (3f).

Prepared from 1-naphthaldehyde (**1f**). Flash column chromatography on silica gel eluting with [hexane (5) : AcOEt (1)] and followed by rechromatography eluting with [hexane (12) : AcOEt (1)] gave **3f** as a colorless crystalline solid: (805 mg, 36 %); mp. 48 °C; $[\alpha]_D^{25} = -25.3^\circ$ (*c* 1.02, CHCl₃); 69 % e.e. [¹H-NMR, with Eu(hfc)₃; δ (OAc) 2.47 (*R*) and 2.51 (*S*)]; IR (KBr) 2250 (C≡N) and 1760 (C=O) cm⁻¹; ¹H-NMR δ 2.18 (s, 3H, OAc), 7.03 (s, 1H, CH), 7.47-7.68 (m, 3H_{arom}), 7.81 (m, 1H_{arom}), and 7.90-8.06 (m, 3H_{arom}); ¹³C-NMR δ 20.46 (CH₃CO), 61.31 (CH), 116.16 (C≡N), 122.59, 125.12, 126.63, 127.00, 127.63, 127.73, 129.19, 130.08, 131.54, 133.95, 169.05 (C=O); MS (70 eV) *m/z* (relative intensity %) 225 (M⁺, 25), 183 (23), 166 (57), 165 (100), 155 (18), 139 (17), 127 (17), and 43 (51); Anal. Calcd. for C₁₄H₁₁NO₂: C, 74.65; H, 4.92; N, 6.22 %. Found: C, 74.78; H, 4.92; N, 6.22 %. The absolute configuration of (-)-**3f** was determined by comparing its optical rotation with that of the authentic sample (*R*)-(+)-**3f** derived from the optically active cyanohydrin (*R*)-(+)-**2f** with a known configuration (*vide infra*).

(R)-(+)-1-Cyano-1-(2-furyl)methyl acetate (3g).

Prepared from 2-furaldehyde (**1g**). Flash column chromatography on silica gel eluting with [hexane (6) : AcOEt (1)] gave **3g** as a colorless oil: (822 mg, 42 %); $[\alpha]_D^{25} = +12.8^\circ$ (c 1.02, CHCl₃); 47 % e.e. [¹H-NMR using Eu(hfc)₃; δ (OAc) 2.56 (*R*) and 2.64 (*S*)]; IR (neat) 2250 (C \equiv N) and 1755 (C=O) cm⁻¹; ¹H-NMR δ 2.16 (s, 3H, OAc), 6.45 (m, 1H, 4'-H), 6.47 (s, 1H, CH), 6.69 (m, 1H, 5'-H), and 7.51 (m, 1H, 3'-H); ¹³C-NMR δ 20.26 (CH₃CO), 55.73 (CH), 111.13, 112.57, 114.16 (C \equiv N), 144.09, 145.04, and 168.75 (C=O); MS (70 eV) *m/z* (relative intensity %) 165 (M⁺, 18), 123 (25), 106 (62), 95 (8), 77 (33), 51 (20), 43 (58), 32 (29), and 16 (100); Anal. Calcd. for C₈H₇NO₃: C, 58.18; H, 4.27; N, 8.48 %. Found: C, 58.45; H, 4.35; N, 8.67 %. The absolute configuration of (+)-**3g** was determined by comparing its optical rotation with that of the authentic sample (*S*)-(-)-**3g** derived from the optically active cyanohydrin (*S*)-(+)-**2g** with a known configuration (*vide infra*).

(S)-(-)-1-Cyano-2-methylpropyl acetate (3h).

Prepared from 2-methylpropanal (**1h**). Flash column chromatography on silica gel eluting with [hexane (6) : AcOEt (1)] gave **3h** as a colorless oil: (328 mg, 15 %); $[\alpha]_D^{25} = -60.6^\circ$ (c 1.19, benzene); 69 % e.e. [¹H-NMR using Eu(hfc)₃; δ (OAc) 2.67 (*R*) and 2.75 (*S*)]; IR (neat) 2250 (C \equiv N) and 1750 (C=O) cm⁻¹; ¹H-NMR δ 1.09 (d, 3H, *J* = 7.0 Hz, CH₃), 1.12 (d, 3H, *J* = 6.8 Hz, CH₃), 2.04-2.28 (m, 1H, CH(CH₃)₂), 2.16 (s, 3H, OAc), and 5.18 (d, 1H, *J* = 5.6 Hz, CH(OAc)); ¹³C-NMR δ 17.32 (CH₃), 17.74 (CH₃), 20.33 (CH₃CO), 31.02 (CH(CH₃)₂), 66.31 (CH(OAc)), 115.98 (C \equiv N), and 169.20 (C=O); MS (70 eV) *m/z* (relative intensity %) 141 (M⁺, 0.3), 99 (78), 81 (17), 57 (67), 43 (100), 41 (35), and 39 (28). Found: *m/z* 141.07701. Calcd. for C₇H₁₁NO₂: M, 141.07891; Anal. Calcd. for C₇H₁₁NO₂: C, 59.56; H, 7.85; N, 9.92 %. Found: C, 59.11; H, 7.85; N, 9.85 %. The absolute configuration of (-)-**3h** was determined by comparing its optical rotation with that of the authentic sample (*R*)-(+)-**3h** derived from the optically active cyanohydrin (*R*)-(+)-**2h** with a known configuration (*vide infra*).

(S)-(-)-1-Cyano-1-hexyl acetate (3i).

Prepared from 1-hexanal (**1i**). Flash column chromatography on silica gel eluting with [hexane (15) : AcOEt (1)] and followed by distillation [bp.

(bath temp.) 128-129 °C / 18 mmHg] gave **3i** as a colorless oil: (680 mg, 27 %); $[\alpha]_D^{25} = -47.5^\circ$ (c 2.064, benzene) [lit.⁷⁹] $[\alpha]_D = +74^\circ$ (c 2, benzene) for *R* isomer with 97 % e.e.]; 75 % e.e. [¹H-NMR using Eu(hfc)₃; δ (OAc) 2.31 (*R*) and 2.34 (*S*)]; IR (neat) 2250 (C \equiv N) and 1750 (C=O) cm⁻¹; ¹H-NMR δ 0.91 (m, 3H, CH₃), 1.20-1.59 (m, 6H, 3 \times CH₂), 1.89 (m, 2H, 2-CH₂), 2.14 (s, 3H, OAc), and 5.31 (t, 1H, *J* = 6.8 Hz, CH); ¹³C-NMR δ 13.78 (CH₃), 20.30 (CH₃CO), 22.24, 24.12, 30.86, 32.13, 61.08 (CH), 116.91 (C \equiv N), and 169.14 (C=O); MS (70 eV) *m/z* (relative intensity %) 169 (M⁺, 0.3), 126 (22), 99 (25), 81 (100), 56 (38), 54 (28), 43 (62), and 41 (61). Found: *m/z* 169.10742. Calcd. for C₉H₁₅NO₂: M, 169.11022; Anal. Calcd. for C₉H₁₅NO₂: C, 63.88; H, 8.93; N, 8.28 %. Found: C, 63.44; H, 8.91; N, 8.25 %.

(R)-(-)-1-Cyano-2-(1-naphthyloxy)ethyl acetate (3j).

Prepared from 2-(1-naphthyl)oxyacetaldehyde (**1j**).⁵⁶ Preparative thin layer chromatography on silica gel developed with [hexane (4) : AcOEt (1)] for 3 times gave **3j** as a slightly red solid: (106 mg, 28 %); mp. 54 °C; $[\alpha]_D^{23} = -30.4^\circ$ (c 1.67, CHCl₃) [lit.⁵⁶] $[\alpha]_D^{23} = +36.1^\circ$ (c 1.19, CHCl₃)

for *S* isomer with 87.4 % e.e.]; 74 % e.e. [¹H-NMR using Eu(hfc)₃; δ (OAc) 2.55 (*R*) and 2.61 (*S*)]; IR (neat) 2250 (C \equiv N) and 1755 (C=O) cm⁻¹; ¹H-NMR δ 2.16 (s, 3H, OAc), 4.41 (d, 2H, *J* = 5.2 Hz, OCH₂), 5.83 (t, 1H, *J* = 5.2, CH), 6.74 (m, 1H_{arom}), 7.35 (m, 1H_{arom}), 7.43-7.55 (m, 3H_{arom}), 7.80 (m, 1H_{arom}), and 8.23 (m, 1H_{arom}); ¹³C-NMR δ 20.33 (CH₃CO), 59.91 (OCH₂), 66.74 (CH), 105.32, 114.91 (C \equiv N), 121.76, 121.94, 125.35, 125.49, 125.85, 126.84, 127.55, 134.55, 153.25, and 168.97 (C=O); MS (70 eV) *m/z* (relative intensity %) 255 (M⁺, 38), 213 (0.3), 194 (0.4), 157 (2), 144 (59), 127 (26), 115 (50), 112 (100), 89 (7), 77 (6), and 43 (87); Anal. Calcd. for C₁₅H₁₃NO₃: C, 70.58; H, 5.13; N, 5.49 %. Found: C, 70.40; H, 5.10; N, 5.23 %.

Stereochemical Correlation of Cyanohydrin Acetates.**Preparation of Optically Active Cyanohydrins.**

In order to determine the absolute configuration of the acetates **3b**, **e**, **f**, **g**, and **h**, optically active cyanohydrins **2b**, **e**, **f**, **g**, and **h** were prepared by the kinetic resolution as shown below.

(R)-(+)-1-Cyano-1-(4-chlorophenyl)methanol (2b).

To an ethereal solution (80 mL) of 4-chlorobenzaldehyde (**1b**, 5.00 g, 35.57 mmol), was added an aqueous solution (40 mL) of KCN (4.63 g, 71.14 mmol) containing tetrabutylammonium bromide (11.6 mg, 0.36 mmol). Concentrated HCl (7.9 mL, 95 mmol) was added dropwise to the mixture with vigorous stirring over a period of 30 min 0 °C under an argon atmosphere and the mixture was stirred at 0 °C for 1.5 h and then at room temperature for 30 min. The ethereal phase was separated, washed with brine (30 mL) and dried (Na₂SO₄). Evaporation gave (±)-**2b** as a colorless oil: (5.82 g, 98 %). The purity of (±)-**2b** was checked by ¹H-NMR and immediately used for the next enzymatic reaction.

To a solution of (±)-**2b** (3.00 g, 17.89 mmol) and isopropenyl acetate (3.58 g, 35.78 mmol) in diisopropyl ether (60 mL), was added lipase from *Pseudomonas* sp. M-12-33 (Amano) (2.00 g), and the suspension was stirred at 40 °C for 47 h under an argon atmosphere. The lipase powder was filtered off and the filtrate was concentrated *in vacuo* to give an oil (3.39 g). ¹H-NMR analysis showed that the resulting mixture contained 4-chlorobenzaldehyde **1b** (9 %), the cyanohydrin **2b** (46 %), and the acetate **3b** (45 %).

tert-Butyldimethylsilyl chloride (1.46 g, 9.66 mmol) was added to a solution of imidazole (1.37 g, 20.13 mmol) in DMF (20 mL) at 0 °C and stirred for 15 min. To this solution, was added the product mixture (3.08 g containing 1.35 g of **2b**, 8.05 mmol), and the mixture was stirred overnight at room temperature. The reaction mixture was quenched with water (60 mL) and extracted with ether (2 × 30 mL). The combined extracts were washed with brine (20 mL) and dried (Na₂SO₄). Flash column chromatography on silica gel eluting with [hexane (20) : AcOEt (1)] gave the *tert*-butyldimethylsilyl ether **13b** as a colorless oil (1.79 g, 38 % from (±)-**2b**): bp. (bath temp.) 120-121 °C / 0.15 mmHg; $[\alpha]_D^{25} = +11.9^\circ$ (*c* 1.06, CHCl₃); ¹H-NMR δ 0.15 and 0.23 (2 × s, 2 × 3H, Si(CH₃)₂), 0.94 (s, 9H, ^tBu), 5.48 (s, 1H, CH), and 7.35-7.47 (m, 4H_{arom}); ¹³C-NMR δ -5.17 and -5.05 (Si(CH₃)₂), 18.19 (SiC(CH₃)₃), 25.54 (C(CH₃)₃), 63.40 (CH), 118.91 (C≡N), 127.49, 129.19, 135.06, and 135.28; Anal. Calcd. for C₁₄H₂₀ClNOSi: C, 59.66; H, 7.15; N, 4.97 %. Found: C, 59.90; H, 7.17; N, 5.14 %.

tert-Butyldimethylsilyl ether **13b** (500 mg, 1.88 mmol) was dissolved in a mixture of concentrated HCl (1 mL), AcOH (2 mL), and water (1 mL), and stirred at 40 °C for 3h. The resulting mixture was

evaporated *in vacuo* to remove AcOH. The residue was extracted with ether (3 × 10 mL) and dried (Na₂SO₄). Evaporation and crystallization from ether/light petroleum gave **2b** as needles (263.6 mg, 84 %): mp. 62 °C; $[\alpha]_D^{25} = +24.3^\circ$ (*c* 1.02, CHCl₃) [lit.⁸⁵] $[\alpha]_D = +27.2^\circ$ (*c* 1.487, CHCl₃)

for *R* isomer]; IR (KBr) 3400 (OH) and 2255 (C≡N) cm⁻¹; ¹H-NMR δ 3.78 (br s, 1H, OH), 5.50 (s, 1H, CH), and 7.40 (m, 4H_{arom}); ¹³C-NMR δ 62.78 (CH), 118.62 (C≡N), 127.99, 129.37, 133.57, and 135.87; Anal. Calcd. for C₈H₆ClNO: C, 57.33; H, 3.61; N, 8.36 %. Found: C, 57.07; H, 3.66; N, 8.33 %.

Acetylation of (*R*)-(+)-**2b** gave (*R*)-(-)-**3b**; $[\alpha]_D^{25} = -36.3^\circ$ (*c* 1.32, benzene); 81 % e.e. [¹H-NMR using Eu(hfc)₃; δ (OAc) 2.70 (*R*) and 2.79 (*S*)].

(R)-(+)-1-Cyano-1-(2-naphthyl)methanol (2e).

Crystallization from ether/hexane afforded fine needles; mp. 117 °C; $[\alpha]_D^{25} = +22.0^\circ$ (*c* 1.02, CHCl₃) [lit.⁸⁵] $[\alpha]_D = +26.4^\circ$ (*c* 0.522, CHCl₃) for *R* isomer with 86 % e.e.]; IR (KBr) 3430 (OH) and 2250 (C≡N) cm⁻¹; ¹H-NMR δ 2.64 (br s, 1H, OH), 5.70 (s, 1H, CH), 7.50-7.62 (m, 3H_{arom}), and 7.81-8.02 (m, 4H_{arom}); ¹³C-NMR δ 63.83 (CH), 118.78 (C≡N), 123.65, 126.20, 126.96, 127.25, 127.81, 128.34, 129.38, 132.42, 132.93, and 133.68; Anal. Calcd. for C₁₂H₉NO: C, 78.67; H, 4.95; N, 7.65 %. Found: C, 78.91; H, 5.02; N, 7.44 %. Acetylation of (*R*)-(+)-**2e** gave (*R*)-(-)-**3e**; $[\alpha]_D^{25} = -16.0^\circ$ (*c* 1.05, CHCl₃); 68 % e.e. [¹H-NMR using Eu(hfc)₃; δ (OAc) 2.80 (*R*) and 2.87 (*S*)].

(R)-(+)-1-Cyano-1-(1-naphthyl)methanol (2f).

Crystallization from ether/light petroleum gave fine needles; mp. 77 °C; $[\alpha]_D^{25} = +9.5^\circ$ (*c* 1.17, CHCl₃) [lit.⁸⁵] $[\alpha]_D = +48.0^\circ$ (*c* 1.325, CHCl₃) for *R* isomer with 73 % e.e.]; IR (KBr) 3380 (OH) and 2250 (C≡N) cm⁻¹; ¹H-NMR δ 3.54 (br s, 1H, OH), 6.02 (s, 1H, CH), 7.37-7.59 (m, 3H_{arom}), 7.71 (m, 1H_{arom}), 7.82-7.88 (m, 2H_{arom}), and 8.02 (m, 1H_{arom}); ¹³C-NMR δ 62.00 (CH), 118.90 (C≡N), 122.89, 125.10, 125.61, 126.46, 127.28, 128.97, 129.90, 130.23, 130.79, and 133.88; Anal. Calcd. for C₁₂H₉NO: C, 78.67; H, 4.95; N, 7.65 %. Found: C, 78.88; H, 4.97; N, 7.64 %.

Acetylation of (*R*)-(+)-**2f** gave (*R*)-(+)-**3f**; $[\alpha]_{\text{D}}^{25} = +5.1^{\circ}$ (*c* 1.17, CHCl_3); 15 % e.e. [$^1\text{H-NMR}$ using Eu(hfc)_3 ; δ (OAc) 2.39 (*R*) and 2.42 (*S*)].

(*S*)-(+)-1-Cyano-1-(2-furyl)methanol (2g).

Optically active TBDMS-ether of cyanohydrin **13g** was prepared from 2-furaldehyde (**1g**) by the same procedure as described for **2b**. Flash column chromatography on silica gel eluting with [hexane : AcOEt = 30:1 to 15:1] afforded *tert*-butyldimethylsilyl ether **13g** as a colorless oil (2.67 g, 48 % from (\pm)-**2g**): $[\alpha]_{\text{D}}^{25} = +9.5$ (*c* 1.02, CHCl_3); $^1\text{H-NMR}$ δ 0.14 and 0.17 ($2 \times \text{s}$, 3H, $\text{Si}(\text{CH}_3)_2$), 0.92 (*s*, 9H, ^tBu), 5.56 (*s*, 1H, CH), 6.40 (*dd*, 1H, $J = 3.4$ and 1.8 Hz, 4'-H), 6.53 (*dt*, 1H, $J = 3.4$ and 0.8 Hz, 3'-H), and 7.45 (*dd*, 1H, $J = 1.8$ and 0.8 Hz, 5'-H); $^{13}\text{C-NMR}$ δ -5.24 (SiCH_3), 18.17 ($\text{SiC}(\text{CH}_3)_3$), 25.45 ($\text{C}(\text{CH}_3)_3$), 58.08 (CH), 109.43, 110.76, 117.23 ($\text{C}\equiv\text{N}$), 143.77, and 148.51; Anal. Calcd. for $\text{C}_{12}\text{H}_{19}\text{NO}_2\text{Si}$: C, 60.72; H, 8.07; N, 5.90 %. Found: C, 60.56; H, 8.33; N, 6.00 %.

Acid deprotection of (+)-**13g** gave **2g** as a colorless oil; $[\alpha]_{\text{D}}^{20} = +23.29^{\circ}$ (neat) [lit.⁵⁷⁾ $[\alpha]_{\text{D}}^{20} = +30.6^{\circ}$ (neat) for *S* isomer]; IR (neat) 3380 (OH) and 2250 ($\text{C}\equiv\text{N}$) cm^{-1} ; $^1\text{H-NMR}$ δ 4.04 (*br s*, 1H, OH), 5.53 (*s*, 1H, CH), 6.40 (*dd*, 1H, $J = 3.4$ and 1.8 Hz, 4'-H), 6.57 (*d*, 1H, $J = 3.4$ Hz, 3'-H), and 7.46 (*dd*, 1H, $J = 1.8$ and 0.8 Hz, 5'-H); $^{13}\text{C-NMR}$ δ 56.79 (CH), 110.19, 110.93, 117.09 ($\text{C}\equiv\text{N}$), 144.35, and 147.44; Anal. Calcd. for $\text{C}_6\text{H}_5\text{NO}_2$: C, 58.54; H, 4.09; N, 11.38 %. Found: C, 58.78; H, 4.27; N, 11.14 %. Acetylation of (*S*)-(+)-**2g** gave (*S*)-(-)-**3g**; $[\alpha]_{\text{D}}^{25} = -14.4^{\circ}$ (*c* 1.05, CHCl_3); 60 % e.e. [$^1\text{H-NMR}$ using Eu(hfc)_3 ; δ (OAc) 2.46 (*R*) and 2.49 (*S*)].

(*R*)-(+)-1-Cyano-2-methylpropanol (2h).

Flash column chromatography on silica gel eluting with [hexane : AcOEt = 8:1 to 5:1] afforded a colorless oil; $[\alpha]_{\text{D}}^{25} = +2.6^{\circ}$ (*c* 1.12, CHCl_3) [lit.⁸⁵⁾ $[\alpha]_{\text{D}} = +2.7^{\circ}$ (*c* 3.908, CHCl_3) for *R* isomer with 17 % e.e.]; IR (neat) 3400 (OH) and 2250 ($\text{C}\equiv\text{N}$) cm^{-1} ; $^1\text{H-NMR}$ δ 1.06 (*d*, 3H, $J = 5.8$ Hz, CH_3), 1.09 (*d*, 3H, $J = 5.6$ Hz, CH_3), 2.04 (*m*, 1H, CH), 3.73 (*br s*, 1H, OH), and 4.28 (*d*, 1H, $J = 6.0$ Hz, $\text{CH}(\text{OAc})$); $^{13}\text{C-NMR}$ δ 17.21 (CH_3), 17.66 (CH_3), 32.97 ($\text{CH}(\text{CH}_3)_2$), 66.87 ($\text{CH}(\text{OAc})$), and 119.33 ($\text{C}\equiv\text{N}$);

Anal. Calcd. for $\text{C}_5\text{H}_9\text{NO}$: C, 60.58; H, 9.15; N, 14.13 %. Found: C, 60.50; H, 9.32; N, 13.97 %. Acetylation of (*R*)-(+)-**2h** gave (*R*)-(+)-**3h**; $[\alpha]_{\text{D}}^{25} = +11.8^{\circ}$ (*c* 1.31, benzene); 14 % e.e. [$^1\text{H-NMR}$ using Eu(hfc)_3 ; δ (OAc) 2.58 (*R*) and 2.65 (*S*)].

SUMMARY

- 1 Enol esters such as isopropenyl acetate were found to be an excellent acylating agent for the transesterification with 1-hexanol catalyzed by lipase in diisopropyl ether. This irreversible transesterification system was successfully applied to the kinetic resolution of racemic 2-halo-1-arylethanols. (*S*)-Esters were obtained at 92-97 % e.e. and (*R*)-alcohols with 80-97 % e.e. were recovered in high chemical yield.
- 2 Stereoselective acylation of racemic mixture of axially dissymmetrical [1,1'-binaphthyl]-2,2'-diol (binaphthol) gave (*R*)-binaphthyl monoesters of 90-95 % e.e. Through the lipase-catalyzed deacylation, alcoholysis, of racemic binaphthyl monoesters, (*R*)-binaphthol was selectively formed at the optical yield higher than 91 % e.e. In combination of acylation and deacylation, both of the enantiomers were obtained in high chemical and optical yields.
- 3 As one of the advantages to conduct the enzymatic reaction in organic solvent, racemic 1-cyano-1-phenylmethanol (mandelonitrile) unstable in aqueous medium was successfully resolved by lipase-catalyzed stereoselective acetylation with isopropenyl acetate. (*S*)-Mandelonitrile acetate with 80-94 % e.e. and the unreacted (*R*)-mandelonitrile with 27-78 % optical purity were obtained in good yields.
- 4 As an extension of this reaction, racemic cyanohydrins were prepared from the corresponding aldehydes and 2-cyano-2-propanol (acetone cyanohydrin) in the presence of cinchona alkaloids, then the racemic cyanohydrins formed were stereoselectively acetylated with isopropenyl acetate in the presence of lipase. As one enantiomer was selectively transformed into acetate, the enantiomer was supplied from the other enantiomer through the addition-elimination equilibrium between cyanohydrin and aldehydes. Under these reaction conditions,

(*S*)-1-cyano-1-(3,4-methylenedioxyphenyl)methyl acetate with 82 % optical purity was obtained even when the chemical conversion of cyanohydrin to acetate reached 91 %.

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LIST OF PUBLICATIONS

The contents of this thesis have been published or will be published in the following original papers.

- 1) "Irreversible and Highly Enantioselective Acylation of 2-Halo-1-arylethanol in Organic Solvents Catalyzed by a Lipase from *Pseudomonas fluorescens*"
Hiratake, J., Inagaki, M., Nishioka, T., and Oda, J. *J. Org. Chem.* **1988**, 53, 6130-6133.
- 2) "Lipase-Catalyzed Stereoselective Acylation of [1,1'-Binaphthyl]-2,2'-diol and Deacylation of Its Esters in an Organic Solvent"
Inagaki, M., Hiratake, J., Nishioka, T., and Oda, J. *Agric. Biol. Chem.* **1989**, 53, 1879-1884.
- 3) "Kinetic Resolution of Racemic Benzaldehyde Cyanohydrin via Stereoselective Acetylation Catalyzed by Lipase in Organic Solvent"
Inagaki, M., Hiratake, J., Nishioka, T., and Oda, J. *Bull. Inst. Chem. Res., Kyoto Univ.* **1989**, 67, 132-135.
- 4) "Lipase-Catalyzed Kinetic Resolution of Racemic Cyanohydrins Formed *in situ* from Aldehydes"
Inagaki, M., Mimura, M., Hiratake, J., Nishioka, T., and Oda, J. *J. Org. Chem.* in preparation.
- 5) "Lipase from *Pseudomonas* sp.: Reactions, Cloning, and Amino Acid Sequence Analysis"
Nishioka, T., Chihara-Shiomi, M., Yoshikawa, K., Inagaki, M., Yamamoto, Y., Hiratake, J., Baba, N., and Oda, J. The Abstract of CEC-GBF International Workshop "Lipases: Structure, Mechanism and Genetic Engineering" Braunschweig, Sept. 13-15, **1990**, p. 50. The Series Book is *in press*.

Other Related Papers

- 1) "Asymmetric Induction in the Base-Catalyzed Reactions Using Polymer-Supported Quinines with Spacer Groups"
Inagaki, M., Hiratake, J., Yamamoto, Y., and Oda, J. *Bull. Chem. Soc., Jpn.* **1987**, 60, 4121-4126.